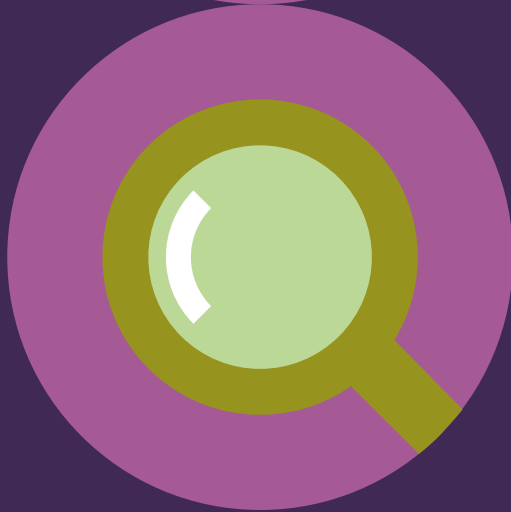


REVIEW OF CONTROLS FOR PATHOGEN RISKS IN SCOTTISH ARTISAN CHEESES MADE FROM UNPASTEURISED MILK

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Glossary of Terms:

ACC	Aerobic colony counts (also known as Total Viable Count, TVC, or Standard Plate Count, SPC)
Affinage	The act or process of ageing cheese
Ageing	The process of holding cheeses in carefully controlled environments to allow the development of microorganisms that usually accentuate the basic cheese flavours. <i>See curing and ripening</i>
AOC	Appellation d'origine contrôlée (French certification granted to certain French geographical indications for wines, cheeses, butters, and other agricultural products)
A _w	Water activity
BAM	Bacteriological analytical manual of the U.S. Food and Drug Administration
Batch	A batch could be cheese produced within a certain vat of which one of more vats on the same day comprise a lot.
BPW	Buffered peptone water
Brining	A step in the manufacture of some cheese varieties where the whole cheese is placed in a salt brine solution. Brining is common in the production of Mozzarella, Provolone, Swiss, Parmesan and Romano cheeses
BTC	Blue type cheese
CCPs	Critical control points
CDC	Centers for Disease Control and Prevention
CFU	Colony forming unit
CPS	Coagulase-positive staphylococci
Curd	Proteinaceous mass precipitated from milk by enzymes or acid/ temperature at the onset of cheesemaking
Curing	The method, conditions and treatment such as temperature, humidity and sanitation, that assist in giving the final cheese product the distinction of its variety. <i>See ageing and ripening</i>
EB	Enterobacteriaceae
<i>E. coli</i>	<i>Escherichia coli</i>
EHEC	Enterohaemorrhagic <i>E. coli</i>
EFSA	European Food Safety Authority
EOP	End of production
FACE network	Farmhouse and Artisan Cheese and Dairy Producers European Network
FBO	Food business operator
FCS	Food contact surface
(US) FDA	United States Food and Drug Administration
FDB	Fat on a dry basis
FDM	Fat in dry matter
FDOSS	Foodborne Disease Outbreak Surveillance System (The CDC's program for collecting and reporting data about foodborne disease outbreaks in the United States)
FSANZ	Food Standards Australia New Zealand

FSIA	Food Safety Authority of Ireland
FSMA	Food Safety Modernization Act (US)
GHP	Good hygiene practice
GMPs	Good manufacturing practices
HACCP	Hazard Analysis and Critical Control Points
HARPC	Hazard Analysis and Risk-Based Preventive Controls
House flora	Microorganisms indigenous to a cheesemaking facility that have a beneficial role in the cheesemaking process
HPA	Health Protection Agency (now Public Health England)
HUS	Haemolytic uraemic syndrome
ICMSF	International Commission on Microbiological Specification for Foods
ISO	International Organization for Standardization
LA	Local Authority
LAB	Lactic acid bacteria
Lactic cheese	Coagulated predominantly by the acidification of the milk rather than by using a large amount of rennet
LC	Lactic cheese
Lot	Legally defined by Codex as “a definitive quantity of a commodity produced essentially under the same conditions” and as “a batch of sales units of food produced, manufactured or packaged under similar conditions” in the UK by the Food (Lot Marking) Regulations 1996.
MC	Microbiological criteria
MDR	Multi-drug resistant
MFFB	Moisture on a fat-free basis
MNFS	Moisture non-fat substance
Mould ripened	Ripening is dominated by moulds either on the rind (e.g. in Brie) or in the paste (such as in a blue cheese)
MPN	Most probable number
NAR	Naladixic acid-resistant
Natural rind	The rind is dominated by natural microflora (moulds and bacteria)
NFCS	Non-food contact surface
NTS	Non-typhoidal salmonella
oPRPs	Operational pre-requisite programs
Paste	The cheese interior beneath the rind
PDO	Protected Designation of Origin
PFGE	Pulsed-field gel electrophoresis
PGI	Protected Geographic Indication
pH	Potential of hydrogen - a measure of acidity (<7) or alkalinity (>7)
PHE	Public Health England (formerly the Health Protection Agency – HPA)
Plate Count at 30°C	A colony count (cfu/ml) which indicates total microbial loading and incubated on a petri dish containing milk agar at 30°C for 3 days (72 hours); (also known as TVC or ACC)

PMO	Pasteurised milk ordinance (set of minimum standards and requirements that are established by the Food and Drug Administration (FDA) for regulating the production, processing and packaging of raw drinking milk)
qPCR	Real time polymerase chain reaction
RH	Relative humidity
Ripening	The chemical and physical alteration of cheese during the curing process. See <i>ageing and curing</i>
RMC	Raw milk cheese
RTE	Ready to eat
Salting	Step in the cheesemaking process requiring the addition of salt to preserve cheese and enhance flavour. May be added while the cheese is in curd form or rubbed on the cheese after it is pressed. Cheese also may be immersed in a salt solution. See <i>brining</i>
SCA	Specialist Cheesemakers Association
SCC	Somatic cell count
SE	Staphylococcal enterotoxins
Secondary fermentation	Secondary fermentation is usually the conversion of citrate to diacetyl, aldehydes and CO ₂ . Primary fermentation is the conversion of lactose to lactic acid.
SF	Sorbitol-fermenting
SFP	Staphylococcal food poisoning
S/M	% Salt-in-moisture
SMP	Salt in the moisture phase
SPC	Standard plate count
STEC	Shiga toxin-producing <i>E. coli</i>
TA	Titrateable acidity
TBX	Tryptone bile x-glucuronide agar
Tempering	Improvement of consistency or resiliency by heating or addition of particular substance(s)
TSB	Tryptic soy broth
TVC	Total viable count or total bacterial count (see plate count at 30°C for definition)
Unhooping	Removal of curd from a mould prior to salting; demoulding
UPC	Uncooked pressed cheese
VFA	Volatile fatty acids
Washed rind	Also called smear-ripened. Cheeses are washed in a 1-3% brine solution to encourage the growth of sticky orange bacteria on the rind
Whey	Liquid remaining after precipitation of curd from milk

Lay Summary and Key Recommendations

This report was prepared for Food Standards Scotland to supply evidence for Scottish artisan cheesemakers and enforcement officials in managing the microbiological safety of artisan cheeses, particularly those produced from unpasteurised milk.

- Chapter 1 examines categorisation of commonly produced cheese types in Scotland and provides an overview of potential critical control points (CCPs) at each stage of the cheesemaking process to control bacterial pathogens of primary concern.
- Chapter 2 analyses currently available predictive models, challenge testing methods and results of challenge testing, providing evidence of the safety, or lack thereof, attained during cheesemaking.
- Chapter 3 provides an analysis of microbiological and physicochemical results obtained from cheesemakers, as well as from the scientific literature and recommendations on testing targets and frequencies to assure process control and production of microbiologically safe products.

The main pathogens of concern posing a risk to the safety of cheeses made from unpasteurised milk are *Listeria monocytogenes*, Shiga toxin-producing *Escherichia coli*, salmonella and *Staphylococcus aureus*.

Contamination of raw milk by pathogens cannot be completely eliminated, despite efforts to control milk hygiene. An approach to risk reduction is therefore recommended to bring Scottish cheeses made from unpasteurised milk to a level of safety equivalent to cheeses made from pasteurised milk. Assuring the safety of cheese made from raw milk is influenced by four primary variables:

- (i) use of raw milk of high microbiological quality with low pathogen contamination levels;
- (ii) the rate and degree of acidification achieved during cheesemaking;
- (iii) the rate of pathogen inactivation during cheesemaking and ageing/affinage;
- (iv) prevention of recontamination from the processing environment or at retail.

A number of factors influencing the microbiological safety of cheese made from unpasteurised milk can be implemented in HACCP plans. These include establishing stringent microbiological criteria for raw milk intended for cheesemaking, improving the microbiological quality of raw milk used for cheesemaking, monitoring trends during cheesemaking and achieving process control, training and education for cheesemakers, proper handling of cheese at retail, prevention of post-process recontamination of cheese, addressing facility issues such as foot traffic and good manufacturing practice (GMP), proper decontamination of brushes used for brining and washing; and environmental control and monitoring of pathogens in the cheesemaking facility.

Microbiological data provided by the Specialist Cheesemakers Association (SCA) showed lower prevalence rates of coagulase-positive staphylococci and generic *E. coli*

in UK raw milk intended for cheesemaking compared to U.S. surveys. No salmonella or *E. coli* O157:H7 were found in 298 and 225 samples analysed. *Listeria monocytogenes* was found in 43 of 639 samples, although this included results of re-sampling after detection. One-on-one technical assistance to artisan cheesemakers incorporating surveillance, testing and process control is recommended to maintain low prevalence rates of pathogenic bacteria in UK raw milk intended for cheesemaking.

A number of predictive models are available to assist cheesemakers in safety assessments by predicting their growth potential in cheese, with the recently developed Australian Raw Milk Cheese Decision Support Tool¹ being the most appropriate and user-friendly model for artisan cheesemakers. Some predictive models may, however, over-predict pathogen growth and survival in cheese. These tools can, however, serve as important guidance, in a first step towards assessment of risk of cheeses made from unpasteurised milk and identification of growth potential of pathogens of concern for shelf life predictions to enhance safety.

It is critically important that microbiological and physicochemical data be routinely monitored to assure that results obtained do not exceed established pre-defined limits. These results are important evidence of process control, which assures safe cheese production for cheesemakers and consumers alike.

Promoting food safety will be central to sustaining growth of the Scottish artisan cheese industry, and to that end, key recommendations are offered as follows;

- Education, training and technical assistance may provide enhanced safety of products manufactured by this important manufacturing sector.
- A survey of the microbiological quality of raw milk specifically intended for artisan cheese production in Scotland may provide an assessment of the overall quality of raw milk used for artisan cheese production and identification of areas where improvements can be made.
- One-on-one technical assistance to artisan cheesemakers incorporating surveillance, testing, and process control is recommended.
- The impact of feeding regimes on microbiological quality of raw milk used for artisan cheese production and effects of feeding dry hay and pasture versus silage and distillers' grains warrants investigation and may reveal sources of contamination that can be mitigated with feed adjustments.

¹ <http://www.foodsafetycentre.com.au/RMCtool.php>

1. Introduction

1.1. Background

There is a growing consumer demand for artisan cheeses worldwide; particularly for those made from raw or unpasteurised milk (Waldman and Kerr 2018). These retain the diverse microbial communities present in milk, giving the product desirable complex flavours and aromas. However, there have been several outbreaks of foodborne illness across the globe that have been linked to the consumption of cheese made from unpasteurised milk, raising concerns about its microbiological safety.

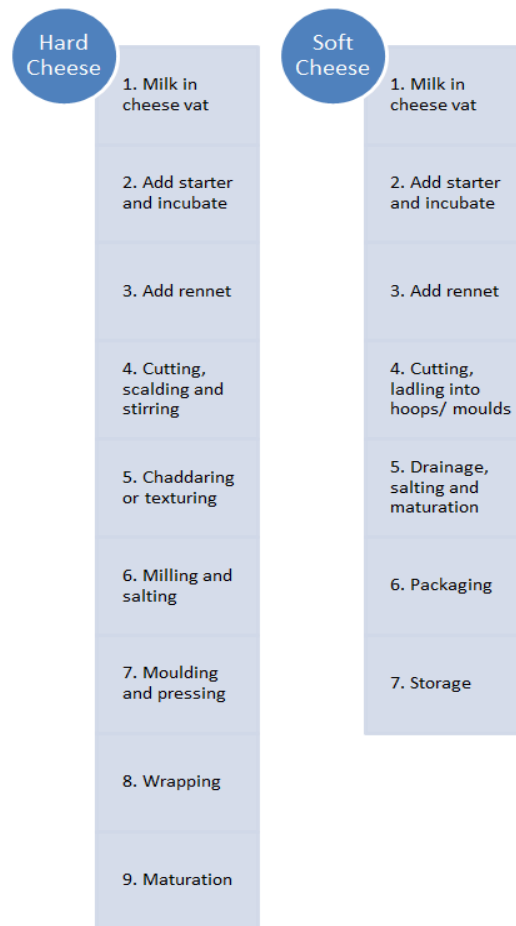
Certain categories of cheeses made from unpasteurised milk, such as fresh soft and soft-ripened varieties, can be considered potentially 'risky' foods because it is possible for pathogenic bacteria to contaminate the final product via contaminated milk from the dairy farm or cross contamination during cheesemaking or post-cheesemaking processes (such as maturation/ripening). As a result, pathogens may grow to levels where they can cause human illness. It is important for cheesemakers to manage microbiological risks during cheesemaking to protect public health.

Raw milk used for cheesemaking can come from any variety of mammalian sources, including cows, sheep, goats, water buffalo, and even camels and reindeer. The breeds, feed, milking cycles and transportation methods dairy farmers choose can all affect the quality and safety of cheese made from raw milk.

In the UK, there have been eight outbreaks of foodborne illness associated with unpasteurised milk cheese since 1983 and 53 outbreaks globally (Yoon et al. 2016; Fox et al. 2017). Of these eight UK outbreaks, six were attributed to *E. coli* O157, one to salmonella and one to *Staphylococcus* spp. In contrast, there have been two outbreaks of foodborne illness in the UK associated with cheeses made from pasteurised milk: one attributed to salmonella and the other to *Staphylococcus aureus*. This review will focus on cheese made from unpasteurised milk. Although not implicated in any major foodborne illness outbreaks connected to cheeses made from unpasteurised milk, *L. monocytogenes* can be isolated from such cheeses and thus should also be considered a significant risk, particularly to susceptible sub-populations with compromised immune systems, including pregnant women and the elderly.

Artisan cheeses produced in the UK are made with varying recipes and techniques producing cheeses from soft, creamy Camembert to firm, farmhouse Cheddar. Despite the variation, the majority of cheese production follows the same fundamental processes (Figure 1).

Figure 1: Simplified cheese making processes for a hard and a soft cheese. Adapted from Appendix 7, SCA Assured Code of Practice (2015)



Many studies have shown the microbiological risk of cheeses made from unpasteurised milk can be reduced by the use of hygienically produced milk of high microbiological quality, proper acidification and ripening (maturation) processes and constant monitoring of the hygiene environments for milk production, cheesemaking and the post-manufacturing stage. Carefully selected time and ripening temperature combinations and acidification processes can prevent the growth of unwanted and potentially harmful bacteria that may cause spoilage and foodborne disease. Some studies have also shown that 60-day ageing can improve the microbiological quality of some cheeses made from unpasteurised milk and this is a legal requirement in the U.S. (Boor 2005). However, other studies have demonstrated that 60-day ageing may not be effective against existing *E. coli* O157 and therefore a risk of foodborne illness may still exist. Foodborne pathogens including *L. monocytogenes*, *Salmonella* spp. and *Staphylococcus aureus* have been shown to be controlled by the naturally occurring bacteriocinogenic lactic acid bacteria (LAB) found in unpasteurised milk; but there has been limited success against Shiga toxin-producing *Escherichia coli* (STEC) (Montel et al. 2014). The interactions between physicochemical conditions (such as pH or water

activity) and natural microflora in the production of cheeses made from unpasteurised milk, and how the resulting conditions impact on the survival and growth of pathogens is not well understood. As current microbiological modelling programmes do not typically take into account the competition between pathogens and unpasteurised milk microflora, it is challenging for cheese producers to demonstrate how pathogens are being controlled through their production process using such programmes.

The main pathogens of concern targeted for control in cheese made from unpasteurised milk are *Staphylococcus aureus*, salmonella, *Listeria monocytogenes*, and STEC. Contamination of raw milk by pathogens cannot be completely eliminated despite efforts to control milk hygiene; therefore cheesemakers must implement a range of strict controls to ensure a safe end product.

For each cheese type produced in Scotland, consideration must be given to the impact of the source of raw milk, including: animal breed, type of feed (dry hay and pasture versus silage), raw milk testing (including milk filters), raw milk handling, storage and transportation, environmental monitoring and control of the cheesemaking environment, cleaning and sanitation and good manufacturing practices (GMPs). When microbiological problems are encountered in cheesemaking, many of the issues relate to lack of process control, or GMPs that are lacking in many artisan cheesemaking establishments (D'Amico et al. 2008).

Regulation (EC) 178/2002² establishes the general principles of food safety and food law, aimed at preventing the marketing of unsafe food and ensuring that systems exist to identify and respond to food safety problems. Article 5 of Regulation (EC) No 853/2004³ requires food business operators (FBOs) to put in place, implement and maintain a permanent procedure based on Hazard Analysis and Critical Control Point (HACCP) principles. HACCP procedures are internationally recognised as useful tools for FBOs to control hazards that may occur in food (EC Commission Notice 2016/C 278/01⁴). A HACCP-based approach to risk reduction is essential to bring Scottish cheeses made from unpasteurised milk to a level of safety equivalent to cheeses made from pasteurised milk.

Assuring the safety of cheese made from raw milk is influenced by four primary variables:

1. Use of high microbiological quality raw milk with low hygiene indicator levels;
2. The rate and degree of acidification achieved during cheesemaking;
3. The rate of pathogen inactivation during cheesemaking and ageing/ affinage;
4. Prevention of recontamination from the processing environment or during retail.

² <https://www.food.gov.uk/business-guidance/general-food-law#regulation-178-2002-provisions>

³ <http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02004R0852-20090420>

⁴ <http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:C:2016:278:FULL&from=EN>

A number of factors influencing the microbiological safety of cheese made from unpasteurised milk can be implemented in HACCP-based risk reduction plans. These include:

- Establishing stringent microbiological criteria for raw milk intended for cheesemaking;
- Improving and maintaining the microbiological quality of raw milk used for cheesemaking;
- Monitoring trends during cheesemaking and achieving process control;
- Training and education for cheesemakers;
- Proper handling of cheese during storage, transportation and at retail;
- Prevention of post-process recontamination of cheese;
- Addressing facility issues such as foot traffic and good manufacturing practices (GMPs);
- Proper decontamination of brushes used for brining and washing; and
- Environmental control and monitoring of pathogens in the cheesemaking facility.

In addition, a number of predictive models are available to assist cheesemakers in making safety assessments by predicting their growth potential in cheese. Most models however over-predict pathogen growth in raw milk cheese. These tools can then serve as important guides, in a first step towards assessment of risk of cheeses made from unpasteurised milk and identification of growth potential of pathogens of concern during shelf life.

Ensuring food safety will be key to sustaining growth of the Scottish artisan cheese industry. Provision of education, training and technical assistance may provide enhanced safety of cheeses produced by this important manufacturing sector. To this end, this report endeavors to summarise existing evidence available on the safe production of raw milk cheese into a consolidated resource for industry and enforcement alike.

1.2. Overall Project Aims

The aim of this project was to conduct a literature review for Food Standards Scotland (FSS) as the basis for improving safety for Scottish artisan cheesemakers producing cheese from unpasteurised milk. Evidence was collated from the scientific literature on controlling factors that can be utilised during the cheesemaking process across different cheese types commonly produced across Scotland, particularly with respect to critical control points (CCPs), the use of predictive modeling, and validation and verification of FBOs processes such as the appropriate use of microbiological testing data.

This project will contribute to delivery of FSS's strategy aimed at reducing the incidence of foodborne illnesses in Scotland⁵.

⁵ http://www.foodstandards.gov.scot/downloads/FSS_Strategy_Doc_Final_2.pdf

The project covered three main areas:

1. Chapter 1: Categorisation of cheese types commonly produced in the UK (e.g. using Codex criteria) and an analysis of critical control points (CCPs) that might be used at various stages of the production of such cheese types through completion of a systematic review of scientific literature on the control of pathogens in cheeses made from unpasteurised milk, supported by industry-captured data. The literature review also examined and compared different methods used to measure physicochemical properties of cheese (pH/titratable acidity, A_w and salt-in-moisture) including recommendations for their suitability and application as evidence of process control at different stages of cheesemaking. This information is summarised in tables detailing CCPs that may be appropriate for the different cheese types, with evidence to support these CCPs. In addition, this literature review provides an overview of factors that affect growth and survival of microbial pathogens in cheese types produced in the UK and provides recommendations to assure continued safe production of UK (and Scottish) artisan cheeses made from unpasteurised milk.
2. Chapter 2: An analysis of currently available predictive modelling and challenge testing methods that are applicable to cheesemakers to enable future recommendations to be made regarding the most suitable methods for individual cheesemakers. In particular, how competitive microflora present in cheese may affect the suitability of predictions, from commonly used models such as ComBase, is addressed.
3. Chapter 3: Analysis of available historical microbiological and physicochemical results obtained from cheesemakers undertaking sampling in their products, as well as guidance offered by international organisations to provide advice to cheesemakers on the examination of trends of microorganisms throughout the cheesemaking process and to inform standardisation of trends.

1.3. Methodology

A literature search was performed using PubMed, Google Scholar and Web of Science in addition to subject-specific databases including AGRICOLA. Search terms including raw milk cheese, pasteurised/unpasteurised cheese, cheese safety, physicochemical parameters, specific individual pathogens (*salmonella*, *E. coli*, *Listeria monocytogenes* and *Staphylococcus aureus*), linked terms such as specific pathogens and cheese, specific pathogens and testing protocols and specific pathogens and raw milk were utilised, along with cross references suggested as a result of the search terms used. Publications yielding data for cheese types not produced in the UK were included in the review where appropriate. Peer-reviewed publications, refereed journal articles, governmental technical reports and published risk assessments were included in the literature review. Recent literature (primarily from the last five years) was highlighted for inclusion, where applicable, with review articles referencing previous literature reviews

included as necessary. Data from the SCA as well as data from unpublished surveys conducted in the U.S. was included to inform assessments of the microbiological status of raw milk used for cheesemaking; evidence for process control, or lack thereof, during cheesemaking; and assessments of cheesemaking facilities for evidence of sources environmental contamination. Over 200 citations appear as supporting evidence for the recommendations made in this report.

2. CHAPTER 1. Categorisation of cheese types commonly produced in the UK (e.g. using Codex criteria) and appropriate critical control points (CCPs) for each stage of the cheesemaking process.

2.1. Cheese Types Produced in the UK (with focus on Scotland): Categorisation using Codex Criteria

The diversity of styles of cheese produced in the UK in general and Scotland in particular, reflects the growing trend of artisan cheese production worldwide, and UK cheesemakers are reviving traditional practices and products. The diverse and complex cheese varieties produced throughout Scotland differ with respect to firmness, milk type, coagulation method, curd cooking temperature, cheese composition and ripening methods. This diversity of cheese types complicates cheese classification for safety assessment. Most traditional classification schemes, such as The Codex General Standard for Cheese (Codex 1978) classify cheeses primarily based on moisture, firmness, fat content and curing characteristics using moisture on a fat free basis (MFFB) and percentage fat on a dry basis (FDB) as primary and secondary descriptors (Table 1). Cheddar cheese, for instance, is a hard, medium to full fat, interior-ripened cheese.

Trmčić et al. (2017) described the challenges associated with the systematic grouping of raw milk cheeses into categories useful in food safety assessments. They proposed use of cheese categorisation to facilitate product assessment for food safety risks and evaluation of interventions for general cheese categories that could be used by cheesemakers to safely and legally produce raw milk cheeses that meet safety requirements equivalent to cheeses made from pasteurised milk. They proposed a cheese categorisation scheme based on pH and water activity (A_w) for assessment of the risk of survival of *Listeria monocytogenes* and other pathogens such as STEC. The authors suggested that because the categorisation scheme proposed was based on measurable properties (pH and A_w) these properties could be used as a standard to meet monitoring requirements. Process controls (active fermentation and acid development) and preventive controls (including sanitation, standard operating procedures (SOPs) and sanitary equipment design), which target prevention of environmental contamination during processing, can help achieve the production of safe products. Trmčić et al. (2017) suggested that this consensus categorisation scheme could provide a scientific foundation to allow assessment of diverse cheese varieties for food safety risks and provide scientifically validated evidence of effective interventions for general cheese categories. To that end, Table 2 lists pH and A_w values for cheese types produced globally, including those produced in Scotland (SCA 2015; Banks 2006) but also assigns the classification proposed by Trmčić et al. (2017).

Criteria of public health significance (A_w , pH and aqueous phase salt) may be far more useful for safety classification than qualitative descriptors used by CODEX. Food

Standards Australia and New Zealand⁶ (FSANZ) notes that it is the combination of these factors, and not a single factor, that achieves food safety.

Table 1: The Codex General Standard for Cheese⁷ (CODEX STAN A-6-1978)

CHEESE TYPES				
Classification of cheese according to firmness, fat content and principal curing characteristics ⁸				
	Term I		Term II	Term III
If the MFFB* is %	The 1st phrase in the designation shall be	If the FDB** is %	The 2nd phrase in the designation shall be	Designation according to principal curing characteristics
<51	Extra hard	>60	High fat	1. Cured or ripened
49-56	Hard	45-60	Full fat	a. mainly surface
54-63	Semi-hard	25-45	Medium fat	b. mainly interior
61-69	Semi-soft	10-25	Low fat	2. Mould cured or ripened
>67	Soft	<10	Skim	a. mainly surface
				b. mainly interior
				3. Uncured or unripened***

* MFFB equals percentage moisture on a fat-free basis, i.e. Weight of moisture in the cheese/Total weight of cheese - weight of fat in the cheese x 100

** FDB equals percentage fat on the dry basis, i.e. Fat content of the cheese/Total weight of cheese - weight of moisture in the cheese x 100

***Milk used for this cheese type is to be pasteurised

⁶<https://www.foodstandards.gov.au/code/proposals/documents/P1007%20PPPS%20for%20raw%20milk%201AR%20SD1%20Cow%20milk%20Risk%20Assessment.pdf>

⁷ <http://www.fao.org/docrep/015/i2085e/i2085e00.pdf>

⁸ <http://www.ianunwin.demon.co.uk/eurocode/foodinfo/codex/cdx-cheesetype.htm>

Table 2: Codex description of different UK cheese styles and factors contributing to microbiological stability (Adapted from SCA Assured Code of Practice 2015; Banks 2006)

Designation	Example Cheese Type	Typical A_w	pH Point of Make	pH Point of Sale
Soft (55% moisture)				
Fresh	Ricotta Mozzarella	0.997 0.987-0.99	5.4-5.8 4.9-5.9	5.4-6.6 (D5*) 5.1-6.2 (E5)
Smear Ripened	Munster	0.922	4.6	5.2
Surface Mold	Brie Camembert	0.972-0.98 0.972-0.98	4.6 4.7	5.5-7.0 (E5) 5.5-7.0 (F5)
Brined	Feta	0.962-0.97	4.3	4.2-5.2
Goat	Chèvre	0.980-0.982	4.1-4.6	4.1-6.7
Semi-hard (44-55% moisture)				
Danish Blue	Danablu	0.945-0.960	4.6	5.2-6.4
Internal Mold Blue	Stilton Roquefort Gorgonzola	0.964-0.970 0.930-0.940 0.940	4.6 4.6 4.7	5.0-6.8 5.5-6.8 6.9 (E2)
Lactic	Crottin	0.972	4.6	4.8-6.2
Pressed uncooked	Gouda	0.920-0.970	5.1	5.1-5.6 (D3)
Hard (20-42% moisture)				
Long aged	Cheddar	0.955-0.970	5.0-5.2	4.9-5.6 (C3)
Pressed short aged	Caerphilly	0.980-0.982	5.3	5.0-5.4
	Cheshire	0.976-0.980	4.7	5.0-5.5
	Lancashire	0.968-0.980	4.9	5.0-5.5
	Wensleydale	0.961-0.980	5.2-5.5	5.0-5.5
Cooked curd	Gruyère	0.947-0.970	5.2	5.5-5.8
	Emmental	0.971-0.980	5.3	5.5-6.0
	Comté	0.958	5.4	5.5

*Designations in parentheses refer to Trmčić et al. (2017) consensus categorisation

Table 2 demonstrates that cheese categorisation and assessment of microbiological risk is complicated beyond simple measurement of pH and A_w , with multiple ways to categorise cheese and no general agreement on how to do this. Almendra-Aliste and Mietton (2014) provide an excellent overview of the complexities associated with cheese classification, characterisation and categorisation, complicated currently by the resurgence of interest in artisan cheese production. While microbiological aspects certainly contribute to the diversity and differentiation of cheese, so does the variability among processing and ageing parameters that influence the chemical composition of cheese and the enzymatic potential (ability to produce enzymes beneficial to cheese quality) during ripening. Fundamental cheesemaking parameters include: acidification rate, time and degree of acidification (which defines casein mineralisation level and moisture loss); the method of milk coagulation (acid versus rennet); additional steps employed during cheesemaking to control moisture loss, such as curd cooking,

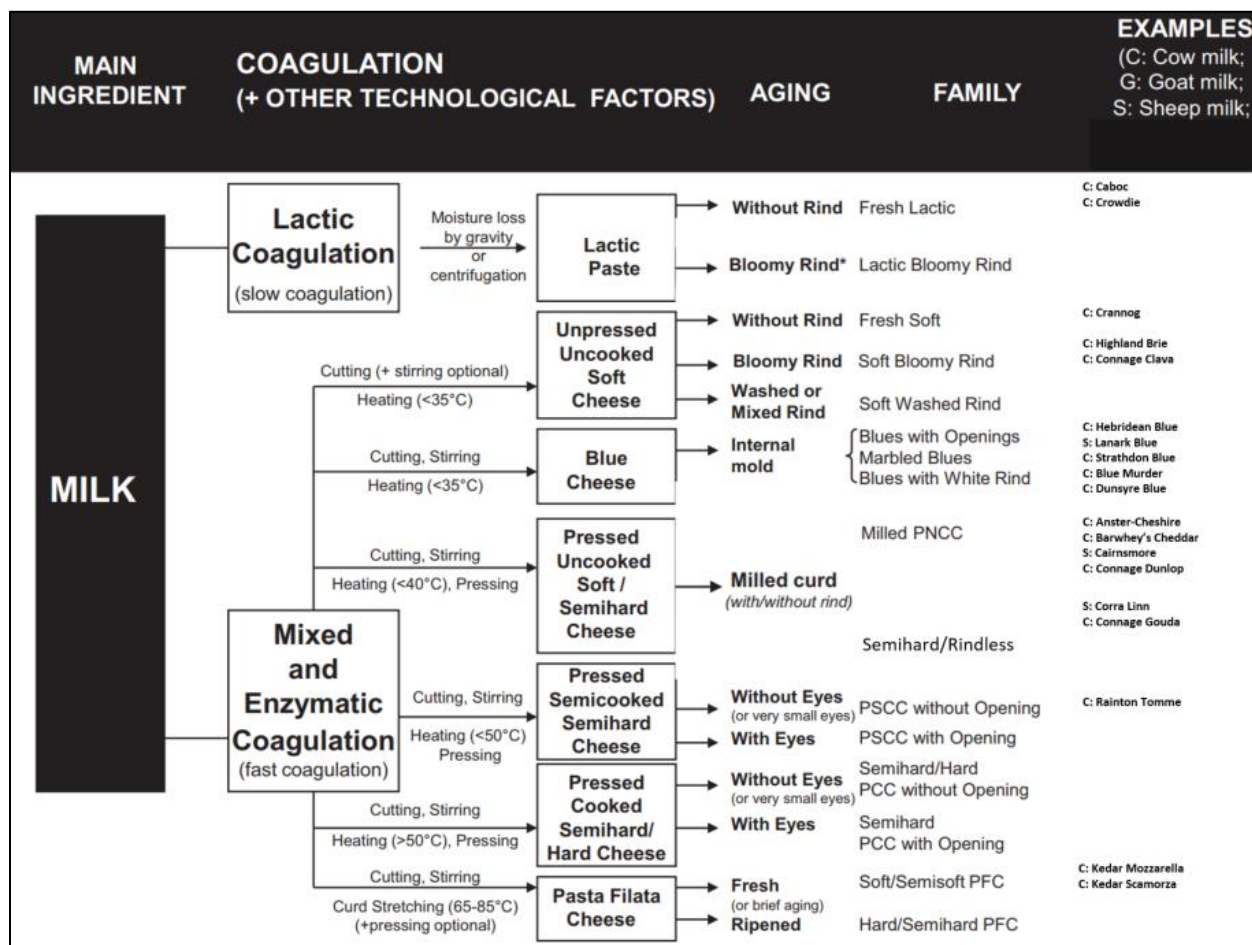
pressing, and salting; and ripening conditions, including temperature, relative humidity, and rates of O₂, CO₂ and NH₃. These fundamental parameters influence the character and diversity of the microbial communities associated with cheese.

There is great variation around the world in the way that cheeses are classified. Almena-Aliste and Mietton (2014) present the argument for an integrative classification approach that more accurately reflects the diversity of cheeses and the differentiation among the many varieties. This becomes further complicated when the European versus Anglo Saxon approaches are considered, as the former classifies cheese based on the technological processes used during cheesemaking, while the latter is primarily based on textural properties. The European approach, influenced largely by France, shows how cheese diversity is primarily due to three key processing parameters: coagulation, draining and ripening. These parameters define the major chemical characteristics of each cheese variety. The type of coagulation (lactic versus enzymatic or rennet) influences the curd structure, firmness and cohesive properties. Whey drainage from curd is achieved through cutting, stirring, and pressing.

Rennet-coagulated cheeses become differentiated by curd cooking temperatures, ranging from uncooked (<40°C), semi-cooked (<50°C), and cooked (>50°C), which are used to contribute to whey expulsion. These processes also affect mineralisation, with rennet-coagulated cooked cheese varieties (such as Alpine style cheeses) having the lowest moisture and highest calcium contents that allow a harder, tighter knit curd, and more durable cheese that permits longer ageing, while the small acid coagulated cheeses have high moisture and a highly de-mineralised structure and are usually consumed fresh. Further transformation occurs during ripening by mould action (external or internal) or development of natural rinds or secondary fermentations. There is confusion regarding soft and semi-soft cheeses because in the French system, soft is reserved for cheese technology that does not involve pressing (Camembert, Brie, Vacherin Mont-d'Or, blue cheeses) versus soft but pressed varieties (Reblochon), which are classified as soft and uncooked pressed cheeses (<35°C).

The microbiological risks that must be controlled during cheesemaking depend greatly on the processing steps and manipulations involved in the production of that specific cheese, therefore alternative classification schemes such as that proposed by Almena-Aliste and Mietton (2014) may be more helpful for conducting safety evaluations because it takes into account all the steps in cheese production. The Codex classification system can be worked from as a starting point but the simplistic system looks only at characteristics of the cheese such as hardness, fat content etc. and fails to consider all the steps where pathogens could be present, introduced, multiply or be reduced. Cheesemakers should be aware of the steps, processes and manipulations within each category (soft, semi-soft etc.) where sufficient controls must be implemented to ensure product safety. Figure 2 shows the diversity of some Scottish cheese styles based upon Almena-Aliste and Mietton's classification.

Figure 2: Scottish Cheese Classification by Technology (adapted from Almena-Aliste and Mietton 2014)



Voysey et al. (2012) state that Cheddar and Cheshire are the most popular hard cheeses in the UK. Of the semi-hard cheeses, Caerphilly and Lancashire are the most popular, and cottage cheese is the most popular soft variety. Williams and Withers (2010) indicated that the approximately 25 Scottish artisan cheesemakers produce 70-80 different farmhouse cheese types, of which one-third are made from unpasteurised milk. Table 3 depicts a breakdown of cheeses currently produced in Scotland (summer 2018) based on the Codex Term 1 descriptors of soft, semi-soft/semi-hard and hard. As of August 2018, of the 64 cheese types being produced in Scotland, 20 (31%) are manufactured from unpasteurised milk from cows, sheep and goats. Twenty-five of the 64 cheese types are hard cheese varieties and 22 are classified as soft. Only 1 (4.5%) of the 22 soft varieties (1.5% overall) are manufactured from unpasteurised milk, versus 12 (48%) of the 25 hard varieties (18% overall) that are made from unpasteurised milk. The information in table 3 may not include all of the cheese varieties produced in Scotland, nor has information on commercial volumes produced been provided. Additionally, many of the listed cheeses have multiple flavoured versions and this information has not been captured.

Table 3. Scottish Artisan Cheese Examples (August 2018) made with pasteurised (P) and unpasteurised (U) milk. Made from cows' milk unless otherwise stated.

Soft	Semi-soft and semi-hard	Hard
Brie/Camembert style Aiket ⁱ (P) Morangie Brie ⁱⁱ (P) Highland Brie ⁱⁱ (P) Arran Mist ⁱⁱⁱ (P) Arran Camembert ⁱⁱⁱ (P) Highland Heart ^{iv} (P) Connage Clava ^{iv} (P) Howgate Brie ^v (P) Creamy Brie ^{vi} (P) Monarch ^{vii} (P)	Blue Fleet Valley Blue ^{xi} (U) Blue Murder ⁱⁱ (P) Scottish Blue Cheese ^{vi} (P) Strathdon Blue ⁱⁱ (P) Dunsyre Blue ^{xv} (U) Arran Blue ⁱⁱⁱ (P) Badentoy Blue ^{vii} (P) Crynoch Blue ^{vii} (P) Howgate Kintyre Brie ^v (P) Hebridean Blue ^{xii} (U)	Cheddar/Cheddar style Tain Truckle ⁱⁱ (P) Isle of Mull Cheese ^{xii} (U) Barweys Cheddar ^{xvi} (U) Loch Arthur Farmhouse Cheddar ^{viii} (U) Cambus O'May ^{xvii} (U) Lochnagar ^{xvii} (U) Auld Reekie ^{xvii} (U) Auld Lochnagar ^{xvii} (U) Lairig Ghru ^{xvii} (U) St Andrew's Farmhouse Cheddar ^{xviii} (U) Border Reiver ^{vi} (P) Vintage Cheddar ^{vi} (P) Granite City ^{vii} (P) Smoked Deesider ^{vii} (P) The Reeds Aricht ^{vii} (P) The Coos R Oot ^{vii} (P) Isle of Kintyre Cheddars ^{xxiii} (P)
Crowdie/Crowdie style Clerkland Crowdie ⁱ (P) Black Crowdie ⁱⁱ (P) Skinny Crowdie ⁱⁱ (P) Caboc ⁱⁱ (P) Knockriach Crowdie ^{xx} (P) Connage Crowdie ^{iv} (P)	Lactic Lady Mary ^{xiii} (P) Grimbister Farmhouse Cheese ^{xiv} (P) Sir Lancelot Cheese ^{xv} (U) – (sheeps' milk)	Cheshire style Anster ^{xviii} (U)
Goats' milk Ailsa Craig ⁱ (P) Glazert ⁱ (P) Nanny McBrie ^{vi} (P)	Gouda style Connage Gouda ^{iv} (P)	Dunlop Traditional Ayrshire Dunlop ⁱ (U/P) Connage Dunlop ^{iv} (P)
Fresh cheese Paddy's Milestone ⁱ (P) Crannog ^{viii} (P) Yester Soft Cheese ^{ix} (P)	Feta style Fet Like ^{vii} (P)	Sheep's milk Corra Linn ^{xv} (U) 4 Ewes ⁱⁱ (P)
Mozzarella style Kedar Mozzarella ^x (P) Yester Mozzarella ^{ix} (P)	Alpine style Rainton Tomme ^{xi} (U)	Caerphilly style Laganory ^{xi} (U) Bewcastle ^{vi} (P)
	Cow's milk cheese Maisie's Keibuck (U)	Goats' milk Bonnet ⁱ (P) Inverloch Goat's Cheddar ^{xxi} (P)
		Cheddar style (made from variety of milk types) Cairnsmore ^{xxii} (U)

(i) <http://www.dunlopdaairy.co.uk/cheese.html>

(ii) <http://www.hf-cheeses.com/>

(iii) <https://www.finecheesemakersofscotland.co.uk/business/bellevue-cheese-company/>

(iv) <http://www.connage.co.uk/>

(v) <https://www.scotchcheese.com/howgate/>

(vi) <http://cowsandco.com/product-category/cheese>

(vii) <http://www.devenickdaairy.co.uk/>

- (viii) <https://www.locharthurshop.co.uk/>
- (ix) <http://www.yesterfarmdairies.co.uk/>
- (x) <http://www.kedarcheese.co.uk/>
- (xi) <http://www.theethicaldairy.co.uk/>
- (xii) <http://www.isleofmullcheese.co.uk/mailorder.htm>
- (xiii) <http://strathearncheese.co.uk/home.html>
- (xiv) <https://www.finecheesemakersofscotland.co.uk/business/grimbister-farm-cheese/>
- (xv) <http://www.erringtoncheese.co.uk/>

- (xvi) <http://www.barwheysdairy.co.uk/the-cheese>
- (xvii) <http://cambusomay.com/>
- (xviii) <https://www.standrewscheese.co.uk/>
- (xx) <http://knockraich.com/home/>
- (xxi) <https://www.scotchcheese.com/inverloch-cheese/>
- (xxii) <https://gallowayfarmhousecheese.co.uk/>
- (xxiii) <https://www.scotchcheese.com/isle-of-kintyre/>

Kocharunchitt, in a report for the Ministry for Primary Industries of New Zealand (MPI 2015) concluded that due to the high degree of variability in the physicochemical parameters among cheeses of the same style, it was not feasible to characterise these cheeses solely according to their style. Almena and Meitton (Almena-Aliste and Mietton 2014) noted that in their experience, qualitative irregularities (e.g. high moisture from incorrect application of salt leading to gassing from coliform growth) observed on ripened cheese are mainly due to a lack of control of cheese composition at unhooping and/or salting, followed by ripening conditions and lastly ripening agents (including environmental contaminants or added microbial cultures).

2.2. Control of Pathogens of Concern and Controlling Parameters During Cheesemaking

2.2.1. Raw Drinking Milk vs. Raw Milk for Cheesemaking

Whilst the sale of raw drinking milk is permitted in England and Wales, the sale of raw drinking milk and cream has been banned in Scotland since 1983 following a number of milk-related illnesses and 12 potentially associated deaths. The introduction of the ban resulted in a marked decline in milk-related illness which has been maintained in subsequent years. In 1995, the Scottish policy was reviewed and following stakeholder consultation, and scientific and medical advice, the ban on raw drinking milk was retained. The ban includes cow, sheep, goats, buffalo and any other species farmed for its milk.

Fluid raw milk and raw milk cheese have different risk profiles. Currently, there are no restrictions regarding the sale of raw milk cheese in Scotland, provided that these products have been produced in compliance with EU food hygiene regulations (Regulation (EC) Nos. 178/2002, 852/2004 and 853/2004⁹).

In contrast, U.S. regulations for use of raw and heat-treated milk in cheesemaking were issued in 1949 (21 CFR Part 133¹⁰). Cheesemakers could select one of two options to assure cheese safety: pasteurise milk used for cheesemaking; or hold cheese at a temperature of more than 2°C for a minimum of 60 days (known as the “60-day ageing rule”). The 60 day holding period recommendation was first published in the 24th August 1950 Final Rule (15 FR 5653) (Boor 2005). The recommendation was established as a

⁹ <http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02004R0853-20141117>

¹⁰ <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=133&showFR=1>

result of expert testimony hearings that included the observation that no disease outbreaks had been associated with cheeses held for more than 60 days.

The science behind the 60-day ageing recommendation remains unclear but was derived from a study that reviewed survival of *Brucella abortus* in Cheddar cheese (Gilman et al. 1946). The study reported that *B. abortus* was not recovered from commercial Limburger cheeses made with *B. abortus* positive milk after the cheeses had been held for 57 days. Test Cheddar cheese made from milk that naturally contained 700-800 cfu/ml *B. abortus* were culture positive for three months. The authors of this study concluded that “an ageing period of 60 days is reasonable assurance against the presence of viable *Brucella abortus* organisms in Cheddar cheese.” However, subsequent research has shown survival of *S. Typhimurium*, *E. coli* O157:H7 and *L. monocytogenes* beyond the mandatory 60-day holding period in Cheddar cheese prepared from unpasteurised milk (Reitsma and Henning 1996). It is not the length of ageing itself, but the physicochemical properties that change during ageing that dictate the safety of or risk posed by a cheese.

In a referral to the U.S. National Advisory Committee on Microbiological Criteria for Foods in April of 1997, the FDA (U.S. Food and Drug Administration) asked if a revision of policy requiring a minimum 60-day ageing period for raw milk hard cheeses was necessary. The FDA noted that such duration may be insufficient to provide an adequate level of public health protection. The FDA cited numerous studies and outbreak investigations documenting the presence of *Listeria*, salmonella, and *E. coli* O157:H7 in raw milk. Of particular concern was the study conducted by Reitsma and Henning (1996) detailing survival of *E. coli* O157:H7 in aged Cheddar cheese. The FDA note, however, that there was “limited epidemiological evidence that foodborne illness results from consumption of raw milk hard cheeses that have been aged for 60 days”. The 60-day rule however has been incorrectly applied to certain cheeses to achieve safety. The U.S. Code of Federal Regulations (21CFR133.182)¹¹ permits manufacture of soft ripened cheeses from raw milk using the 60-day ageing rule to assure safety, and raw milk cheeses that have not been properly aged are illegal in the U.S. – this includes all raw milk cheeses – even surface mold ripened soft cheeses such as Brie and Camembert style cheeses.

Due to renewed interest in artisan cheeses, artisan producers are manufacturing soft mould ripened cheeses from raw milk, using 60 days of ageing to achieve safety, a practice that increases *Listeria* risk due to its ability to grow to high population levels during 60 days of refrigerated storage (Ryser and Marth 1987; D'Amico et al. 2008).

2.2.2. Microbial Safety of Cheeses made from Raw Milk

The main pathogens of concern in unpasteurised milk posing a risk to the safety of cheeses are *Listeria monocytogenes*, enteropathogenic *Escherichia coli*, particularly O157:H7, salmonella and *Staphylococcus aureus*. Table 4 summarises the key

¹¹ <https://www.gpo.gov/fdsys/granule/CFR-2012-title21-vol2/CFR-2012-title21-vol2-sec133-182>

characteristics of these four main pathogens. Of these, *Listeria monocytogenes*, enterotoxin production by coagulase positive staphylococci and salmonella are considered the most significant microbiological hazards associated with cheese (FACEnetwork 2016). Challenges posed by these pathogens in cheesemaking have been comprehensively reviewed (e.g. FSANZ 2009¹², D'Amico and Donnelly 2017, Fox et al. 2017) and additional findings are summarised here.

Table 4: Summary of characteristics of the major pathogens found in raw milk cheeses

	<i>Listeria monocytogenes</i> ¹³	STEC - Shiga toxin-producing <i>Escherichia coli</i> ¹⁴	<i>Salmonella</i> spp. ¹⁵	<i>Staphylococcus aureus</i> ¹⁶
Microbiology	Gram-positive, non-spore-forming, facultatively anaerobic rods	Mesophilic, Gram-negative rod-shaped (Bacilli) bacterium	Facultative anaerobic Gram-negative rods	Toxin producing, Gram-positive, catalase positive cocci. Can grow aerobically but are capable of facultative anaerobic metabolism
Growth temperature range	Optimum growth 30-37°C. May grow at temperatures below 0°C or up to 45°C	7-50°C (Optimum 37°C)	5.2-46.2°C (Optimum 35-43°C)	Growth at 7-48°C, optimum 37°C. Production of Staphylococcal enterotoxins (SEs) occurs at 10-48°C (optimum 40-45°C)
Growth pH	4.3-9.5, but may survive at lower pH	4.4-9.0 (Optimum 6.0-7.0)	3.8-9.5 (optimum 7.0-7.5)	For growth 4.0-10.0 (optimum 6-7). For SE production 4.0-9.6 (optimum 7-8)
Growth A_w	Optimum 0.97; growth range=0.90-0.97; survival at 0.81	Optimum 0.995; minimum 0.95	Optimum 0.99; range 0.93-0.99. Survival has been shown in high fat-low moisture foods	Optimum 0.98; range 0.83-0.99
Infectious dose	Regulation (EC) 2073/2005 permits a level of 100 cfu/g in a RTE food, however the infectious dose is considered to vary depending on the strain and susceptibility of the host. Illness	Not known, considered to vary depending on the strain and susceptibility of the host but some studies have shown the dose to be <100 organisms	The infective dose can vary depending on the strain, the immunocompetence of the individual and the nature of the food. Data from foodborne outbreaks suggest that infections may be caused by the	Not known. Amount of toxin necessary to cause illness depends on susceptibility of person however studies have shown as little as 1µg of SE can cause illness

¹²

<https://www.foodstandards.gov.au/code/proposals/documents/P1007%20PPPS%20for%20raw%20milk%201AR%20SD3%20Cheese%20Risk%20Assessment.pdf>

¹³ <https://www.fsai.ie/listeriamonocytogenes.html>

¹⁴ http://www.foodsafety.govt.nz/elibrary/industry/Escherichia_Coli-Organism_Invades.pdf

¹⁵ www.fsai.ie/salmonellaspecies.html

¹⁶ <https://www.fsai.ie/staphylococcusaureus.html>

	has occurred at lower doses ¹⁷		ingestion of as few as 10-45 cells	
Reservoir/ source	Widely distributed throughout the environment. Humans and various animals can also act as a reservoir	Found in the guts of ruminant animals. Cattle are considered primary reservoirs but sheep and deer may also carry the organism	<i>Salmonella</i> spp. reside in the intestinal tract of humans and warm-blooded animals. They are shed in the faeces. Hooves, hair and skin of animals can become contaminated as they walk, sit or lie in faecally contaminated ground or litter	Occurs on skin and mucous membranes of most warm-blooded animals including food animals and humans
Mode of transmission and commonly associated foods	Chilled, ready-to-eat foods including smoked fish, pâté, and unpasteurised cheeses	Faecal-oral person-to-person transmission. Foods involved in previous outbreaks include hamburgers, salads, bean sprouts, raw milk and cheese	<i>Salmonella</i> spp. may be spread during slaughter. Eggs, poultry meat, milk and chocolate have all been identified as vehicles of transmission	Commonly found in foods of animal origin such as raw meat and raw milk. Survives well in the environment where it may become part of the flora of the processing equipment and act as a source of contamination
Incubation period	3-70 days, but commonly 2-3 weeks	Typically 3-4 days but may range from 1-21 days	12-36 hours	1-7 hours
Control measures	Separate ingredients (raw milk) and finished, ready to eat cheeses, follow good manufacturing practices, ensure facility is sanitary and adequately designed, ensure cleaning and sanitation methods are effective and conduct environmental pathogen monitoring	GHP and GMP at all stages in the food chain, i.e. at farm level, milking shed, manufacturing, processing, catering, retail etc. Implementation of a HACCP based food safety management system including process control i.e. temperature control and storage. Test against microbiological criteria (e.g. generic <i>E. coli</i>) as appropriate when validating and verifying the HACCP plan	GHP and GMP at all stages in the food chain, i.e. at farm level, manufacturing, processing, catering, retail etc. Particular attention should be paid to the prevention of cross contamination. Implementation of a HACCP based food safety management system including process control i.e. temperature control and storage. Test against microbiological criteria as appropriate when validating and verifying the HACCP plan	Avoid the use of raw materials that may be contaminated with high numbers of <i>S. aureus</i> . Ensure food handlers are aware of the importance of GHP, particularly hand washing. Implementation of a HACCP based food safety management system including process control i.e. temperature control and storage. Test against microbiological criteria as appropriate when validating and verifying the HACCP plan

¹⁷ <https://www.cdc.gov/listeria/outbreaks/ice-cream-03-15/index.html>

Gould et al. (2014) examined outbreaks of foodborne illness submitted to the U.S. Center for Disease Control and Prevention (CDC)'s Foodborne Disease Outbreak Surveillance System (FDOSS) between 1998-2011 involving cheese as a vehicle of infection. Of 90 outbreaks identified, 38 (42%) outbreaks involved cheese made with unpasteurised milk, 44 outbreaks (49%) involved cheese made with pasteurised milk and the pasteurisation status was not reported for the other eight (9%). *Salmonella* (34%), *Campylobacter* (26%), *Brucella* (13%) and Shiga toxin-producing *E. coli* (11%) were the causative agents most frequently involved in outbreaks involving cheese made from unpasteurised milk. For outbreaks involving cheese made from pasteurised milk, norovirus (39%) and *Listeria monocytogenes* (24%) were reported most frequently. Norovirus contamination of cheese likely results from infected food handlers (Tuan Zainazor et al. 2010). In 10 outbreaks, queso fresco and salmonella were the common cheese/pathogen pairs. Another six outbreaks showed pasteurised queso fresco and *Listeria* as common cheese/pathogen pairs. Queso fresco is a fresh, un-ripened high-moisture soft cheese that lacks barriers to pathogen growth and poses a risk to public health if contaminated by microbial pathogens that can grow to high levels in this product. Most outbreaks are due to cheese manufactured by unlicensed manufacturers using raw milk (Gould et al. 2014); this cheese can be safely produced by using pasteurised milk provided that controls are in place to prevent environmental recontamination during production.

Although cheeses have been associated with documented outbreaks of foodborne illness, epidemiological evidence collected from around the world confirms that outbreaks are an infrequent occurrence. However the outbreaks that do occur can have serious consequences (Johnson et al. 1990; Altekruuse et al. 1998; Gould et al. 2014; Trmčić et al. 2017; Donnelly 2004). Fox et al. (2017) reported that since 1980, 53 outbreaks of foodborne illness due to cheese consumption have occurred over a timespan where production of 250,000,000 tonnes of both raw and unpasteurised cheese occurred.

Cheesemaking is a centuries-old process originally designed as a way to preserve raw milk *via* fermentation. Through process manipulations that select for beneficial microflora in raw milk, such as lactobacilli, streptococci and lactococci, or direct addition of these organisms as starter cultures, microbial communities form in cheese and in certain varieties create conditions that suppress the growth of bacterial pathogens. However, cheeses may become contaminated with pathogens due to their presence in the raw milk and survival during the cheesemaking process. Bacterial pathogens may also contaminate cheese *via* post-processing contamination if sanitation and other measures in the processing plant or at retail food establishments, where cutting and wrapping takes place, are not sufficient to prevent re-contamination (Heiman et al. 2016; Johnson et al. 1990; Sauders and D'Amico 2016). The characteristics of the specific cheese variety dictate the potential for growth and survival of microbial pathogens, and in general, ripened high-moisture soft cheeses present a higher risk for growth and survival of pathogens compared with aged, hard cheeses where a

combination of factors including pH, salt content, and A_w interact to achieve microbiological safety.

Factors contributing to the safety of cheese with respect to bacterial pathogens include milk microbiological quality, starter culture or native lactic acid bacterial growth during cheesemaking, pH, salt, control of ageing conditions and associated chemical changes. Soft cheeses are more likely to be involved in cheese-associated outbreaks of foodborne illness than hard and semi-hard cheese. During epidemiological investigations, compositional data (e.g. pH, salt and moisture) of cheese involved in outbreaks is rarely provided but could reveal important information regarding causative factors, including lack of process control that is essential to assure cheese safety (Fox et al. 2017).

2.2.3. Shiga toxin-producing *Escherichia coli* (STEC)

2.2.3.1. General Characteristics

Escherichia coli are Gram-negative, facultatively anaerobic, rod-shaped bacteria that comprise part of the normal intestinal flora of humans and other warm-blooded animals, and are commonly found in soil and water¹⁸. Some strains, however, can cause disease. Of particular concern to cheese producers are the Shiga toxin-producing *E. coli* (STEC), named for their ability to produce the cytotoxins Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2) or both (Montet et al. 2009; Marozzi et al. 2016; Farrokh et al. 2013), with additional subtypes Stx2a to Stx2g with varying virulence also described (Venegas-Vargas et al. 2016).

Both VTEC (verocytotoxigenic *E. coli*) and STEC (Shiga toxin-producing *E. coli*) are terms used synonymously throughout the scientific literature to describe these strains, with STEC now being the preferred term. VTEC was used by Konowalchuk et al. (1977) in reference to the effect of toxins on Vero cells (African green monkey kidney cells) in tissue culture, while STEC denotes that toxins are similar to toxins produced by *Shigella dysenteriae* (Shiga-like) (Chart 2000). Shiga toxins are encoded by the *stx* gene. Genes encoding *E. coli* virulence factors are either located on plasmids, on pathogenicity islands (large 10-200 kb genome regions), or on integrated bacteriophages (Hacker and Kaper 2000) all of which enable a phenomenon known as horizontal gene transfer, allowing transfer of genetic material between organisms.

STEC are responsible for a range of human infections, from mild watery diarrhoea to haemorrhagic colitis that may be complicated by haemolytic uraemic syndrome (HUS). Enterohaemorrhagic *E. coli* (EHEC), such as *E. coli* O157:H7, are a subset of the STEC which cause more severe clinical symptoms and potentially high mortality (Venegas-Vargas et al. 2016). The *eae* gene, present on the locus of enterocyte effacement (LEE) pathogenicity island, encodes the intimin protein which is important for attachment to the intestinal mucosa (Venegas-Vargas et al. 2016). Strains possessing the LEE island and at least one *stx* subtype are classified as EHEC (Venegas-Vargas et al. 2016). Severe disease has been epidemiologically linked to the presence of Stx2 (Gamage et al. 2004), and strains that possess the Shiga-toxin 2 gene (*stx2*) and *eae* (intimin production) or *aaiC* plus *aggR* genes are associated with a higher risk of severe illness (EFSA 2013). However, there is no clear consensus as to what defines a “pathogenic STEC”. In the UK, the detection of any isolated *E. coli* with *stx* genes would be considered as potentially pathogenic and necessitate action to be taken when detected in a ready to eat food such as cheese, as defined in the UK draft STEC policy position¹⁹. Sorbitol-fermenting (SF) *E. coli* O157 has recently emerged as an important cause of outbreaks and sporadic infections in Europe (Jaakkonen et al. 2017).

¹⁸ <http://www.foodstandards.gov.au/publications/Pages/agentsoffoodborneill5155.aspx>

¹⁹ [http://www.foodstandards.gov.scot/downloads/Draft UK Working Policy on Detection of STEC in Food August 2016 FSS v 1 - 12 August 2016.pdf](http://www.foodstandards.gov.scot/downloads/Draft%20UK%20Working%20Policy%20on%20Detection%20of%20STEC%20in%20Food%20August%202016%20FSS%20v%201%20-%2012%20August%202016.pdf)

E. coli O157:H7 was first characterised in 1982 during epidemiological investigations of two outbreaks that occurred in North America. The majority of *E. coli* O157:H7 cases are sporadic in nature, although many cases, often characterised by bloody diarrhoea, HUS and kidney failure, have been traced to the consumption of raw milk (Borczyk et al. 1987; Martin et al. 1986) with additional cases in England linked to yogurt made from pasteurised milk (Morgan et al. 1993).

2.2.3.2. STEC Reservoirs

Cattle are the principal reservoir of STEC. Locking et al. (2006) found that 25% of cases occurring in Scotland in 2004 were reported in persons living or working near farms. In U.S. outbreak investigations where food was identified as the vehicle of transmission, minced beef is the product most frequently linked to human illness (Erickson and Doyle 2007). However, only 20% of *E. coli* O157:H7 infections occurring in Scotland between 1999 and 2008 were outbreak-related, the remainder were sporadic cases making identification of vehicles extremely challenging (Locking et al. 2011).

Strachan et al. (2001) showed that *E. coli* O157:H7 could be contracted from the environment in proximity to animal reservoirs. In follow-on studies, these authors reviewed three primary *E. coli* O157:H7 transmission routes: foodborne, environmental, or person to person contact (Strachan et al. 2006). They analysed *E. coli* O157:H7 outbreaks in Scotland that occurred between 1994 and 2003 and found that 40% of outbreaks were foodborne, 54% were environmental and 6% involved both routes. The authors noted that from 1999 to 2003, 64% of all *E. coli* O157:H7 outbreaks included secondary cases, highlighting the significance of person-to-person spread. The largest outbreaks (by size) were foodborne, representing 83% of outbreak-associated cases. Mapping studies of the Grampian region of Scotland showed a positive association with indicators of cattle and sheep density. These authors found that the incidence of *E. coli* O157:H7 illness in children between the ages of 1-4 years living in rural areas was three times greater than for children living in urban areas, postulating that more frequent exposure to farm animals and their faeces and increased likelihood for hand to mouth transfer of pathogens could be risk factors. The Monte Carlo simulation conducted by these authors showed the environmental risk of *E. coli* O157:H7 infection to be 100 fold greater from visiting a pasture compared to risk from minced beef consumption. These studies affirm the critical importance of controlling environmental contamination on the farm and in the food manufacturing environment.

The concentration and frequency of shedding of *E. coli* O157:H7 by cattle varies greatly among individual animals. “Super-shedders” are cattle that shed concentrations of *E. coli* O157:H7 at levels greater than 10^4 colony-forming units (cfu)/g in faeces (Munns et al. 2015; Stein and Katz 2017; Murphy et al. 2016; Chase-Topping et al. 2008). *E. coli* O157:H7 isolates from super-shedders share a commonality with isolates linked to human illness (Munns et al. 2015) and human outbreaks during summer/early Autumn were correlated with the seasonal effects associated with shedding (Vugia et al. 2007). Super-shedders have been reported to have a substantial impact on the prevalence and

transmission of *E. coli* O157:H7 in the environment, being responsible for up to 96% of bacteria shed by all animals in some studies (Omisakin et al. 2003). Ternent et al. showed shedding prevalence rates of 23% for herds and 7.9% for cattle for *E. coli* O157:H7 on Scottish farms (Toft et al. 2005).

The recto-anal junction (RAJ) was identified as the primary site of *E. coli* O157:H7 colonisation of cattle, and may be involved in super-shedding (Naylor et al., 2003; Davis, 2006, Cobbold, 2007). Schurman et al. (2000) found cattle colonised by 26 different EHEC serotypes. Faecal shedding was found to be seasonally dependent in a U.S. study, with 80% of feedlot cattle shedding in the summer versus only 5-10% shedding in the winter (Naumova et al. 2007), but this does not appear to be the case in the UK (Henry et al. 2017). Unknown factors are responsible for super-shedding but may be due to characteristics of the bacterium, such as acid resistance (Diez-Gonzalez et al. 1998), animal host factors, diet and the environment. Super-shedding is sporadic and inconsistent, possibly suggesting intermittent sloughing from biofilms of *E. coli* O157:H7 colonising the intestinal epithelium in cattle. Phenotypic and genotypic differences have been noted in *E. coli* O157:H7 recovered from super-shedders (Munns et al. 2015) with evidence to support differences in the faecal microbiome between super-shedders and low-shedders. If super-shedders could be easily identified, strategies such as bacteriophage therapy, probiotics, vaccination, or dietary inclusion of plant secondary compounds (such as tannins) could be specifically targeted at this subpopulation (Munns et al. 2015). Matthews et al. (2013) modelled the effects of vaccinating super-shedding cattle and showed a significant (50-85%) reduction in the risk of transmission of *E. coli* O157:H7 to humans if the vaccine was effective.

Murphy et al. (2016) conducted a 12 month longitudinal study in two Irish dairy herds to identify the STEC O157 and O26 shedding status of animals and the impact on raw milk (although it was not stated if the milk was then intended for pasteurisation or not). Dairy herd owners participated in the study voluntarily. Recto-anal swabs, raw milk, milk filters, sand and water samples were tested from each herd. For virulence determination, real time PCR (qPCR) was applied to extracted DNA. Four common virulence genes of STEC O157 and O26 were targeted (*stx1*, *stx2*, *eae* and *hlyA*). Although four super-shedding animals were identified, no STEC O157 or O26 were recovered from raw milk, milk filters or water samples following adherence to normal recommended practices for milk production. One O26 super-shedding animal was identified, which was colonised by both O157 and O26. A survey was administered to the farm owners regarding farm practices, and methods for control. When a positive result was obtained, verbal advice was provided on personal hygiene and best practice to prevent the dissemination of STEC on the farm. The authors suggested that enforcing sanitation rules, including the use of disinfectants at key points and wearing protective clothing and footwear promotes good hygiene during milking and can prevent milk contamination by STEC, even when harvested from super-shedding animals.

Venegas-Vargas et al. (2016) conducted a cross-sectional study of STEC shedding in dairy and beef cattle herds in Michigan. STEC was found to be more prevalent in beef cattle (21%) versus dairy cattle (13%). Factors significantly associated with STEC shedding in dairy cattle included:

- maximum average temperatures exceeding 28.9°C, 1-5 days prior to sampling;
- animals in their first lactation;
- animals less than 30 days in lactation.

The authors suggested that possible control strategies could be considered for animals in their first lactation and/or within the first 30 days of lactation. Daily cleaning of cattle feeders reduced risk of STEC shedding compared with feeders cleaned less frequently.

There is a poor understanding of the dynamics and transmission of STEC virulence in dairy herds and farm environments. The lack of data to support the mathematical modelling of virulence factor spread, persistence, or evolution in farm environments is a major obstacle in the development of predictive tools to assess STEC virulence transmission (Lambertini et al. 2015). As such, Lambertini et al. (2015) explored the occurrence and dynamics of four *E. coli* virulence factors (*eae*, *stx*₁, *stx*₂, and γ -*tir*) on three U.S. dairy farms over an eight year period that spanned 2004-2012. The authors extracted DNA and determined the presence and relative abundance of the four virulence factors.

Shiga toxins were found to be nearly ubiquitous on the three study farms. A low prevalence of virulence factors was found to be associated with milk, (up to 1.9% for *stx* and 0.7% for γ -*tir*) but not milk filters (up to 35% for *stx* and 20% for γ -*tir*). These findings suggest that STEC harbouring these virulence factors, or free DNA encoding virulence genes, are concentrated in the filters and more likely to be detected as opposed to in milk where they are diluted. Feed and trough water were less likely to harbour virulence factors when compared with faecal and composite manure samples. *eae* was detected in all water categories (drinking water, trough water and on-farm streams).

The authors indicated that well water is unlikely to be a vehicle introducing STEC into farms, most likely due to protection from contamination by faecal material. Trough water had a consistently higher prevalence of STEC virulence factors than source (well) water, documenting that water can act as a reservoir and vehicle for cow-to-cow pathogen spread. Due to low sample numbers, the authors consider the feed data preliminary, but the data shows lower levels of virulence factors in finished feed versus feed ingredients (haylage, silage and corn). The distribution of *E. coli* classes was highly skewed toward NLNS (non-LEE non-STEC *E. coli*; negative for all four tested *E. coli* virulence factors), in 85-95% of milk samples. Higher prevalence of virulence factors in milk filters compared to bulk milk highlights the impact of sampling strategies and assay sensitivity on observed prevalence. No consistent seasonality was observed across

study farms over the 7 to 9 year study period and the authors did not find a correlation between seasonal effects and presence of virulence factors.

A longitudinal study of *E. coli* dissemination on four Wisconsin dairy farms identified contaminated animal drinking water as the most probable vehicle for infection of animals and a potential intervention point for on-farm control of dissemination of this pathogen (Shere et al. 1998).

Lambertini et al. (2015) noted the challenges associated with direct cultural identification of pathogenic *E. coli* due to the wide diversity of *E. coli* subtypes in manure and faeces. Aside from *E. coli* O157:H7, isolating STEC strains is confounded by lack of metabolic differences that can be utilised for their discrimination. The isolation of *E. coli* O157:H7 from manure and faecal samples requires labour intensive extraction with immunomagnetic beads and use of expensive chromogenic agars. By using qPCR to detect four virulence factor genes associated with enteropathogenic *E. coli*, the authors were able to conduct direct semi-quantitative comparison of the relative abundance of virulence factors within the *E. coli* community associated with the analysed sample. The authors cautioned that this cannot predict with certainty the presence of a specific pathogenic serotype but implies its possible presence. Virulence factor patterns consistent with *E. coli* O157:H7 were not detected in any milk samples, and only 0-2% of milk filter samples, confirming that even when STEC and EHEC are present in cow faeces, appropriate sanitary practices effectively lower the risk of milk contamination. The authors concluded “eradication of pathogenic *E. coli* on dairy farms still appears to be a far-fetched goal due to the high prevalence” but noted that understanding the ecology of STEC can lead to improved strategies to control pathogenic *E. coli* on farms.

2.2.3.3. UK Policy Position on STEC and Legal Requirements

The draft UK policy position on STEC²⁰ considers the presence of STEC in food to be confirmed when one or more *stx* genes are detected in an isolated *E. coli* strain. The presence of STEC in a ready-to-eat (RTE) food (termed “food profile 1”) is considered a serious risk to public health (UK Working Policy, Food Standards Scotland 2016). Food profile 2 refers to foods intended to be consumed following a treatment that will remove STEC risk. The European Food Safety Authority (EFSA) (2013) concluded “*Strains positive for Shiga-toxin 2 gene (stx2)- and eae (intimin production)- or [aaiC (secreted protein of EAEC) plus aggR (plasmid-encoded regulator)] genes are associated with higher risk of more severe illness than other virulence gene combinations. The 2011 STEC O104:H4 outbreak demonstrated the difficulty of predicting the emergence of “new” pathogenic STEC types by screening only for the eae gene or by focusing on a restricted panel of serogroups. A molecular approach utilising genes encoding virulence characteristics additional to the presence of stx genes has been recommended*”.

²⁰[http://www.foodstandards.gov.scot/downloads/Draft UK Working Policy on Detection of STEC in Food August 2016 FSS v 1 - 12 August 2016.pdf](http://www.foodstandards.gov.scot/downloads/Draft%20UK%20Working%20Policy%20on%20Detection%20of%20STEC%20in%20Food%20August%202016%20FSS%20v%201%20-%2012%20August%202016.pdf)

In the U.S., enterohaemorrhagic *E. coli* (O157:H7) and certain non-O157:H7 STECs are considered adulterants in cheese by the FDA and cheeses contaminated with STEC are not permitted in commerce. The FDA notes that samples that are only positive for *stx1* and/or *stx2* are indicative that non-O157 STEC may be present. They caution that since there are ~ 300 serotypes of STEC and not all appear to cause severe illness in humans, the isolated STEC requires further testing²¹. The U.S. National Advisory Committee on the Microbiological Criteria for Foods is currently addressing the question of “what defines or differentiates an STEC as a human pathogen from other STEC that are under-represented in severe illnesses²²”.

2.2.3.4. STEC Outbreaks from Milk Sources

In a review of outbreaks occurring in England and Wales during 1992-2000, it was reported that *E. coli* O157:H7 was the most common cause of milk-borne infectious disease (Pennington 2014). Of nine outbreaks occurring during this time period, five of the outbreaks were attributed to consumption of unpasteurised milk, one to pasteurised milk that had been mixed with unpasteurised milk and three of the outbreaks to milk sold as pasteurised. Small dairies bottling their own milk were cited as posing a significant problem.

A 1994 outbreak in West Lothian in Scotland, that affected 100 individuals, with 24 hospitalisations and one death, was linked to pasteurised milk. Matching isolates were recovered from 69 patient stool samples, a section of pipeline connecting the pasteuriser and the milk bottling equipment, raw milk from a bulk carrier from a farm supplying the dairy and from bovine faecal samples from the implicated farm (Pennington 2014).

A second outbreak linked to pasteurised milk was reported in North Cumbria in England (Pennington 2014). Between late February and early March 1999, 114 individuals were affected, with 88 having culture confirmed *E. coli* O157:H7 infection. Three children developed HUS and 28 individuals were hospitalised. Milk came from a farm comprised of 65 animals. Although pulsed-field gel electrophoresis (PFGE) patterns matching clinical strains were not isolated from milk samples, matching isolates were recovered from straw bedding, floors of animal pens, slurry samples and faecal samples from 11 animals. The farm pasteuriser had been given a warning a year prior to the outbreak and faulty pasteurisation was cited as a factor leading to this outbreak. New heat exchanger plates had been installed by the farmer a few days prior to the outbreak, but there were no tests to confirm correct functioning of the pasteuriser. There were additional failures associated with the pasteuriser (flow diversion) as well as inadequate temperature monitoring.

²¹ <https://www.fda.gov/downloads/Food/ComplianceEnforcement/FoodCompliancePrograms/UCM456592.pdf>

²² <https://www.fsis.usda.gov/wps/wcm/connect/fsis-content/internet/main/topics/data-collection-and-reports/nacmcf/current-subcommittees/nacmcf-subcommittee-stec-2015-2017>

Operational prerequisite programs (oPRPs) to prevent introduction of faecal contamination into the raw milk supply are the primary control of STEC contamination. Appropriate verification to assess effectiveness of hygienic practices on prevention of raw milk contamination is therefore recommended (FACEnetwork 2016). The SCA recommends monitoring of raw milk for *E. coli* and coliforms, with targets of <100 cfu *E. coli*/ml.

2.2.3.5. STEC in Cheese

Challenge studies have shown that *E. coli* O157:H7 can grow at temperatures as low as 7°C in milk (King 2014) and has been shown to survive during refrigerated storage in a variety of fermented dairy products. Despite this, the incidence of *E. coli* O157:H7 in cheese appears to be quite low. For example, Bowen and Henning (1994) failed to recover *E. coli* O157:H7 in 50 U.S. retail samples of cheese that consisted of American types (Cheddar, Colby and Monterey Jack) and non-American types (Swiss, Mozzarella, Edam and Muenster). Similarly, no *E. coli* O157 was detected in 153 soft and semi-soft cheeses made with raw cows', ewes' and goats' milk in a survey conducted in Belgium (Vivegnis, 1999). Williams and Withers (2010) failed to detect *E. coli* O157:H7 in a 2010 survey of 28 artisanal farmhouse cheeses manufactured in Scotland.

In an analysis of the U.S. FDA's Domestic and Imported Cheese Compliance Program results²³ from January 1, 2004 and December 31, 2006, 3 (0.09%) positive samples for EHEC were found out of 3,360 cheese samples tested (D'Amico and Donnelly 2011). Positive products consisted of imported Mexican-style soft cheese and imported soft-ripened cheese from Honduras (D'Amico and Donnelly 2011). This low incidence in cheese is in contrast to reports of isolation of STEC from 25.5% of beef samples in Argentina (Brusa et al. 2012). In a follow-on FDA study (FDA 2016), no *E. coli* O157:H7 were recovered from 1,606 tested cheese samples (473 domestic and 1133 imported), the majority (63%) of which consisted of semi-soft cheeses (Fontina, Gouda and Provolone). STEC was however found in 11 of 1,606 samples (0.68% positive), and 1 of the 11 positive samples (0.06%) contained the "top 6" serotype O111:H8. In France, *E. coli* levels in raw milk can be stricter (with some businesses aiming for <10 cfu/g) and the results of this study with regards to imported cheeses should be considered with that in mind. The cheese sample that tested positive was a hard raw goats' milk cheese produced in the Midwestern U.S. The FDA found non-compliant²⁴ levels of generic *E. coli* (>10 MPN/g and <100 MPN/g) in 87 of 1,606 samples tested. Of the 87 non-compliant samples, 18 were U.S. domestically produced cheeses while 69 were imported samples. Using Pearson's chi-squared test, no evidence of an association between the presence of generic *E. coli* and the pathogens salmonella, *L. monocytogenes*, *E. coli* O157:H7 or STEC was found (FDA 2016). The FDA concluded that while detection of *E. coli* may be useful in assessing facility hygiene and potential

²³ <http://www.foodprotection.org/files/food-protection-trends/Apr-11-DAmico.pdf>

²⁴ <https://www.fda.gov/downloads/Food/ComplianceEnforcement/FoodCompliancePrograms/UCM456592.pdf>

loss of process control, levels of *E. coli* should not be used to directly predict the presence of pathogens, i.e. conferring the safety of food.

Vernozy-Rozand et al. (2005b) evaluated the prevalence of STEC in 1,039 retail raw milk cheeses (produced by both large scale cheese plants and small scale farm houses) across France by colony hybridisation and characterised the STEC strains isolated by virulence genes and serotypes. The cheese types sampled included soft, hard, unripened and blue mould cheeses; specifically, the most important cheese types tested were farm white mould rinded soft cheeses, farm uncooked hard cheeses, farm washed rinded soft cheeses and industrial white mould rinded soft cheeses (Table 5).

Table 5: Summary of results from evaluation of prevalence of STEC French retail raw milk cheeses (modified from Vernozy-Rozand et al. 2005b)

Cheese type	No. of cheeses tested	No. of <i>stx</i> -positive STEC isolates
Industrial washed ripened soft cheese	42	7 (16.7%)
Farm washed rinded soft cheese	132	11 (8.3%)
Industrial white mould rinded soft cheese	96	10 (10.4%)
Farm white mould rinded soft cheese	399	46 (11.5%)
Industrial uncooked hard cheese	88	21 (23.9)
Farm uncooked hard cheese	184	33 (17.9%)
Industrial unripened cheese ²⁵	13	0 (0.0%)
Farm unripened cheese	22	4 (18.2%)
Industrial blue mould cheese	44	1 (2.3%)
Farm blue mould cheese	8	2 (25%)
Other farm cheese	11	1 (9.1%)

While 16.7% of industrial washed rinded soft cheese tested positive for *stx*, only 8.3% of farm washed rinded soft cheeses were *stx*-positive showing that in this instance, farmhouse cheeses can be safer than industrially produced products. While 23.9% of industrial uncooked hard cheeses were *stx*-positive, only 17.9% of samples of farm uncooked hard cheeses were positive. The majority of isolates (19 strains) belonged to the O6 serogroup and the other strains belonged to the O174, O175, O176, O109, O76, O162 and O22 serogroups. No isolates belonged to the O serogroups most frequently isolated from French patients with hemorrhagic colitis or HUS (O157:H7). One strain had the *eae* gene; while the *eae* gene is carried by the majority of non-O157:H7 STEC strains (Vernozy-Rozand et al. 2005), one isolate had this additional virulence factor suggesting the human pathogenic potential of strains isolated during this study. The authors advised that considering the wide distribution of STEC on dairy farms, strategies should focus on establishing educational programmes to bring about an awareness of STEC issues among dairy farmers, cheesemakers and consumers.

In Europe, in addition to STEC serotype O157:H7, STEC serotypes O26:H11, O103:H2, O111:H8, and O145:H28, many others²⁶ have been previously associated with illness

²⁵ The authors do not specify whether this is a hard or soft cheese.

(Perrin et al. 2015; Nobili et al. 2016). The serotype O26:H11 is the second leading HUS-causing serotype worldwide (after O157:H7) and is found in dairy products such as cheeses made from unpasteurised milk. A small number of HUS cases identified each year in France and Ireland are caused by serotype O26:H11 (Bonanno et al. 2017; Murphy et al. 2016). As per the UK policy position, FSS/FSA regard any STEC as potentially pathogenic, and do not recognise specific serotypes as more or less pathogenic.

Madic et al. (2011) cautioned that the hypothetical loss of *stx* genes during isolation of STEC from foods could result in tested food being considered safe and free from contamination by pathogenic STEC. These authors, in an examination of 265 samples from soft and smear semi-hard uncooked cheeses made from raw cows' milk and 135 samples from unpasteurised goats' milk cheeses observed that *stx*-negative *E. coli* O26:H11 were isolated from *stx*-positive cheese samples, suggesting bacteriophage-associated *stx* gene loss during enrichment or isolation procedures. *Stx* phage induction²⁷ is known to result in STEC lysis and release of new *stx* phages particles. This phenomenon could negatively impact STEC screening in foods based on *stx* gene detection by qPCR alone (Madic et al. 2011).

Bonanno et al. (2017) evaluated the influence of physicochemical parameters related to the cheesemaking process on the induction rate of *stx* phages from STEC O26:H11, including H₂O₂, NaCl, lactic acid and temperature. In addition, selective agents from the analytical STEC enrichment and detection procedure (XP CEN ISO/TS 13136) were tested including novobiocin, acriflavin, cefixim-tellurite, and bile salts. An impact of H₂O₂ and NaCl on *stx* phage induction was observed. Production of *stx* phages was also observed during a real cheesemaking process. By contrast, no significant effect could be demonstrated for the chemical agents on the STEC detection procedure when tested separately, except for acriflavin and novobiocin, which reduced *Stx*1 phage production in some cases.

In conclusion, these results suggest that the cheesemaking process might trigger the production of *stx* phages, potentially interfering with the analysis of STEC in the finished product. These authors demonstrated that oxidative (aeration and exposure to oxygen) and salt stress, which are both likely to occur during cheesemaking, had the ability to induce *stx* phages *in vitro*. Additionally, production of *stx* phages was also observed during cheesemaking when milk was inoculated with a strain of STEC O26:H11. Because of these difficulties, the UK requires *stx* to be detected in isolated, viable cells of *E. coli*. These observations suggest that *stx* phages could be present as free particles in cheeses and could infect other *E. coli* or enterobacterial species from the microflora in the cheese matrix or inside the human gut after consumption – a potential, but unconfirmed hazard. These free *stx* phages could also contribute to the production of

²⁶ http://www.hps.scot.nhs.uk/utility/fullimage.aspx?imageurl=../images/weekly_report/2017/1732/ecolisurv_table4.gif

²⁷ *Stx* phages have a phage cycle similar to bacteriophage. In the lysogenic state, the *stx* phage DNA is integrated into the STEC chromosome and the expression of *stx* phage genes, is inhibited. *Stx* prophage induction in STEC results in production of phage particles and *stx* and thus relates to virulence.

stx-positive signals obtained during PCR-based screening of STEC in foods, explaining the reported difficulties in isolating STEC from *stx*-positive food samples. Voysey et al. (2012) reported that it was difficult to separate STECs from cheese curds and difficult to find STEC when lactic acid bacteria are present.

2.2.3.6. STEC Outbreaks associated with Raw Milk Cheese

STEC have been implicated in a number of cheese-related outbreaks occurring around the globe. Gould, et al. (2014) reported that between 1998 and 2011, STEC caused 11% of outbreaks from cheese made with unpasteurised milk. Reid (2001) reported three outbreaks of *E. coli* O157:H7 infection in Scotland, occurring between 1994-1999, involving the consumption of cheese made from unpasteurised milk. Despite acceptable hygienic conditions, milk storage temperatures at two dairies were found to be inadequate to prevent pathogen growth. At one facility, no starter culture was being used as it was not required (which would have reduced the pH), and in another facility, the maturation step was insufficient to achieve pH reduction and decrease bacterial populations of concern.

Between 26th October 2002 and 1st February, 2003, an outbreak of *E. coli* O157:H7 illness occurred in Alberta, Canada (Honish et al. 2005). The outbreak was linked to consumption of unpasteurised Gouda cheese manufactured on a farm. A total of 13 cases with the same outbreak PFGE profile were reported. Cases ranged in age from 22 months to 77 years. Ten cases reported bloody diarrhoea, and HUS developed in two patients who were 22 months and 4 years of age. Cheese samples from intact packages wrapped at the plant tested positive for *E. coli* O157:H7 of the same outbreak PFGE profile 104 days after production. The cheese was in compliance with microbiological and ageing requirements as set out in the company's HACCP; samples from each lot of cheese had been analysed for microbiological quality (generic *E. coli* and *S. aureus*) under the supervision of the provincial regulator, prior to identification of the outbreak. Cheese lots were subject to positive release. The lot found to be positive for *E. coli* O157:H7 104 days after production had provided a satisfactory *E. coli* result of 40 cfu/g, which is well below the SCA's recommended limit of <100 cfu/g.

Espie et al. (2006) reported on an outbreak of *E. coli* O157:H7 involving three family members who had reported consuming fresh goats' cheese in France. Although strains matching clinical isolates were not recovered from any tested cheese, inspections of the mixed species farm that produced the goats' cheese revealed inadequate hygienic conditions for cheese manufacture. Manual milking, environmental contamination and lack of basic hygiene provided potential for cross-contamination between unpasteurised milk and faecal matter during milking, or at a later point during cheese preparation and assembly. The owner of the farm was required to implement appropriate corrective actions, including use of strict hygienic practices during milking, cheese production and husbandry along with separation of animal species on the farm, and use of tap water in animal troughs.

In 2010, aged unpasteurised Gouda cheese contaminated with *E. coli* O157:H7 caused an outbreak of illness in the U.S. that affected 41 individuals (McCollum et al. 2012). Deficient sanitation practices and insufficient cheese ageing times were found during inspections of the manufacturing facilities. The business failed to conduct microbiological testing on raw milk used for cheesemaking, and violations of GMP were observed. Despite finding the outbreak strain of *E. coli* O157:H7 in Gouda cheese, the definitive contamination source was not identified. The FDA conducted inspections of the cheesemaking facility suspected of causing this multistate outbreak in the U.S. in 2010 (FDA 2011)²⁸. Deficiencies reported included the failure of the cheesemaker to conduct adequate hand washing during cheesemaking; lack of effective cleaning and sanitising procedures as evidenced by the presence of mud, manure, straw and wood chip debris on the cheese room floor; and failure to minimise contamination from milking and outdoor activities through use of outer garments that prevent manure contact with foods and food contact surfaces. *E. coli* O157:H7 isolated from aged cows' milk cheese wrapped in chestnut leaves produced by this facility was indistinguishable from outbreak strains collected by public health officials in Oregon and Washington State.

Cardosa and Marin (2017) reported on post-process recontamination of Mozzarella cheese most likely from a food worker during production. Non-O157 STEC strains were isolated from cheese during a sampling time period coincident with presence a farm employee who worked on the production line and was later dismissed from the company (although the dismissal was for unknown reasons). 15 samples were collected every 6 months for 2 years, however, all the STEC strains isolated were from the cheese samples obtained in the second collection in January 2005. Non-O157 STECs were absent from cheese collected during all other time periods, when the worker was not present, indicating the worker's potential involvement in the contamination process.

As STEC strains can survive or grow during cheesemaking, particularly in soft cheeses, a stochastic quantitative microbial risk assessment (QMRA) model was developed to assess the risk of HUS associated with the five main pathogenic serotypes of STEC in raw milk soft cheeses (Perrin et al. 2015). A baseline scenario represents a theoretical worst-case scenario where no intervention was considered throughout the farm-to-fork continuum. The impact of seven pre-harvest scenarios (vaccines, probiotics, milk farm sorting²⁹) on the risk-based level was expressed in terms of risk reduction. The impact of the pre-harvest interventions ranged from 76% to 98% risk reduction, with highest values predicted for scenarios combining a decrease of the number of cows shedding STEC and of the STEC concentration in faeces. The impact of post-harvest interventions on the risk-based level was also tested by applying five microbiological criteria (MC) at the end of ripening. The five MC differed in terms of sample size, the number of samples that may yield a value larger than the microbiological limit, and the

²⁸ <https://wayback.archive-it.org/7993/20161023101020/http://www.fda.gov/ICECI/EnforcementActions/WarningLetters/2011/ucm260500.htm>

²⁹ Milk farm sorting - exclusion of farms repeatedly delivering raw milk containing the highest concentration of *E. coli* among the farms tested.

analysis methods. The reduction in predicted risk from the baseline scenario (theoretical worst case scenario with no interventions across the entire farm to fork continuum) varied from 25% to 96% by applying microbiological criteria without pre-harvest interventions (which include vaccination, probiotics, antimicrobials, bacteriophages, sodium chlorate, alteration of diet, exclusion of highly contaminated milk) and from 1% to 96% with combination of pre- and post-harvest interventions, showing that there are a number of strategies that can be used to achieve STEC risk reduction in raw milk soft cheese. Among these, exclusion of farms repeatedly delivering high levels of *E. coli* in milk resulted in an 87% predicted risk reduction, showing the benefits of focus on milking hygiene and reduced fecal contamination of teats as a key defense against STEC.

In conclusion, STEC contamination of cheese can best be prevented through focus on milk hygiene and prevention of faecal contamination of milk and cheese. While surveys document a low prevalence of STEC in tested cheese, challenge testing studies show potential for survival of STEC during manufacture of a wide range of cheese types. Research is needed to identify and eliminate the vehicles introducing STEC to dairy cattle in order to reduce on-farm prevalence and improve the safety of cheese manufactured from unpasteurised milk. Outbreak investigations have revealed instances of lack of basic hygiene including sanitation and cleaning deficiencies in both farm and cheese operations, failure to minimise cheese contamination from milking and outdoor activities, and lack of adequate hand washing.

2.2.4. Salmonella

2.2.4.1. General Characteristics

Salmonella enterica is a Gram negative bacterial species comprising more than 2,600 serovars (types). Salmonella is present in the gastrointestinal tracts of all warm blooded animal species, including humans. The majority of salmonella cases are foodborne and, as explained in a comprehensive report³⁰ issued by the USDA's Economic Research Service, salmonella contamination can occur in a wide range of animal and plant products, and raw milk can be a source of salmonella, most likely due to faecal contamination from the herd. Most salmonella species associated with human disease belong to subspecies I and consist of typhoidal and non-typhoidal serovars (Gal-Mor et al. 2014). Non-typhoidal salmonella (NTS) serovars such as Typhimurium and Enteritidis have broad host specificity, and approximately 93.8 million cases of NTS salmonellosis occur worldwide each year. NTS transmission to humans typically occurs via contaminated poultry, eggs and dairy products. *Salmonella* spp. incidence rates reported in the U.S. for raw milk range from 0 to ~9% (Jayarao and Henning 2001).

O'Donnell (1995) examined 1,673 samples from bulk tank milk in England and Wales and found 0.36% positive for salmonella. Wells et al. (2001) examined recovery of salmonella from faecal samples obtained from dairy cows in 91 herds from 19 U.S. states and *Salmonella* spp. was recovered from 5.4% of the samples. Recovery rates from cows on farms with less than 100 animals were much lower (0.6%) than those from farms with over 100 cows, where recovery rates were 8.8%. The incidence of *Salmonella* spp. in milk is expected to occur at a much lower frequency than in faecal samples. Most farmstead cheesemakers maintain small dairy herds, where the lower incidence data would be likely to apply. The SCA reported no detection of *Salmonella enterica* in 298 raw milk samples submitted by UK cheesemakers between January 2011 and August 2012 (SCA, 2015). Similarly, no salmonella was detected in 234 samples of raw milk intended for the production of raw milk cheese collected over two manufacturing seasons in Vermont (D'Amico et al. 2008; D'Amico and Donnelly 2010). Williams and Withers (2010) did not detect salmonella in a 2010 survey of 28 artisanal farmhouse cheeses manufactured in Scotland.

However, despite this, *Salmonella enterica* serovars Enteritidis, Typhimurium and Dublin have been associated with foodborne disease outbreaks involving raw milk and milk products. *S. enterica* serotype Typhimurium definitive type (DT) 104 emerged in the UK as an important source of human infection in the late 1980s (Threlfall et al. 1996). Subsequent outbreaks of human illness traced to dairy sources were reported in the U.S. in Vermont, Nebraska, California (Cody et al. 1999) and Washington State (Villar et al. 1999). This particular organism is notable because it possesses resistance to multiple antibiotics. Two outbreaks of *S. enterica* serovar Typhimurium DT104 infection were linked to consumption of Mexican-style soft cheese manufactured from raw milk in Northern California (Villar et al. 1999; Cody et al. 1999). Aceto (2000) conducted a

³⁰ https://www.ers.usda.gov/webdocs/publications/43984/52807_eib140.pdf?v=0

survey to assess the herd prevalence of *S. Typhimurium* DT 104 in Pennsylvania dairy herds and of the 51 farms surveyed, 11 were positive for *Salmonella* spp. and 4 were positive for *S. Typhimurium*, two of which were DT-104 positive. *S. enterica* serovar Dublin is present in dairy cattle and was identified as the most invasive of the salmonella bacteria for humans in studies conducted in Denmark (Lester et al. 1995).

Salmonella spp. can grow readily in acidic environments with growth at pH 3.7 reported, but the minimal pH in which growth is observed varies depending on acid type, temperature, available oxygen, growth medium, level of inoculation and serovar (El-Gazzar and Marth 1992). Many strains can also grow at low temperatures, although growth is typically inhibited at <5°C. Overall, outbreaks of salmonellosis associated with the consumption of cheese are often attributed to the use of raw or inadequately pasteurised milk from an infected herd (El-Gazzar and Marth 1992; Johnson et al. 1990; Cody et al. 1999; Gould et al. 2014) or non-compliance with good manufacturing practices and inadequate control programs (Fontaine et al. 1980). Despite NTS being the leading cause of bacterial gastroenteritis in France, dairy products are not recognised as important vehicles for salmonella infection (Dominguez et al. 2009) suggesting the need for improved surveillance systems and systematic typing of strains to identify outbreaks and their likely sources.

2.2.4.2. Fate of Salmonella in Cheesemaking

D'Amico et al. (2014) validated the process lethality associated with traditional cheesemaking procedures for Gouda cheese in order to assess whether current manufacturing parameters yield a level of microbiological safety equivalent to pasteurisation, and whether multi-drug resistant (MDR) strains of salmonella behave similarly to non-resistant (non-MDR) salmonella. The most resistant microorganisms of public health significance (NACMCF 2006) likely to present a public health risk in raw milk Gouda cheese include the multidrug resistant (MDR) strains *Salmonella enterica* serovar Typhimurium var Copenhagen DT104 and *Salmonella enterica* serovar Newport. When inoculated to raw milk at initial population levels of approximately 20 cfu/ml, counts increased significantly to 734 cfu/g on day 1, followed by significant decreases over 60 days of ageing to levels of <1 cfu/g on day 60 when examined by direct plating. Through enrichment culture however, viable cells remained detectable by enrichment for 210 ±40 days. The results of this study indicate similar behavior of MDR and non-MDR salmonella in Gouda cheese, and MDR status does not enhance survival of MDR strains in Gouda cheese.

In comprehensive risk assessments of the manufacturing processes used for Swiss style and Italian Grana cheeses where the curd is cooked at high temperatures for a relatively long time, FSANZ determined that heating of curd to high temperatures coupled with aging to reduce moisture rendered a level of control equivalent to cheese made from pasteurised milk³¹ and is the principal control of safety in these cheeses.

³¹<https://www.foodstandards.gov.au/code/proposals/documents/P1007%20PPPS%20for%20raw%20milk%201AR%20SD3%20Cheese%20Risk%20Assessment.pdf>

The dryness as the cheese ages and salt level prevent growth of organisms re-contaminating the cheese. In the absence of a lethal heating or curd cooking step, microbial safety in cheese relies on other hurdles where microbiological control is achieved by the combined and prolonged exposure to stresses incurred during the manufacture and ageing process. Changes in pH, acidity, salt, moisture, oxidation-reduction potential, and osmotic and oxidative stress interact to create an environment hostile to microbial pathogens, thereby achieving microbiological safety in cheeses such as Gouda.

In studies of Feta made from unpasteurised ewes' milk (Papadopolou et al. 1993), a reduction in pH, moisture, and water activity combined with increasing salt concentration during 15 days of brine storage achieved a 10,000 fold decrease in populations of *S. enteritidis*. A 7 log decrease of *S. Enteritidis* over the 90-day ripening period of Savak Tulum (a traditional Turkish cheese) was reported, attributable to significant changes in pH, acidity and water activity (Calicioglu 2009). *Salmonella* counts decreased significantly following drying and vacuum packaging of cheese over the 60 day ageing period for all treatments from ~734 cfu/g to <1 cfu/g for all strains combined.

2.2.4.3. Salmonella Outbreaks Associated with Raw Milk Cheese

Maguire et al. (1992) reported on an outbreak in England and Wales caused by *Salmonella* Dublin associated with an Irish soft cheese made from unpasteurised milk. *S. Dublin* was cultured from 9 of 15 cheese samples obtained from the manufacturer, along with cheese curd from four batches of cheese. Screening of the milking herd revealed four cows were shedding *S. Dublin*. For the manufacture of cheese (by the same small farm based business), unpasteurised cows' milk was incubated at 30°C with starter culture for 1 hour, rennet was added, and the milk was allowed to set for 30 min before the curd was cut. Curds were cooked at 34-35°C, and then the product was matured in a curing room on open wooden shelving for 12-21 days. The firm ultimately decided to continue manufacturing the cheese from pasteurised milk to ensure product safety.

Dominguez et al. (2009) reported on an outbreak of *Salmonella enterica* serovar Montevideo infection in 23 individuals that occurred in France in between 2006-2007. Strains matching patients were isolated from a raw milk soft cheese. The plant producing the cheese produced 3,600 kg of cheese/day for distribution to supermarket chains throughout the country. Microbiological analysis was conducted by taking samples of six cheeses from each batch produced each week and results revealed the presence of *Salmonella* Montevideo in cheeses produced on the 15th September, 2006. One farm supplying the cheese plant had *Salmonella* Montevideo detected in bulk tank milk. The outbreak went undetected until January 2007. This outbreak illustrates the challenges associated with routine testing to detect contamination, due to the non-homogenous distribution of pathogens. Testing does not assure safety, which is why producers must rely on GMPs and hygienic practices to ensure safety.

2.2.5. *Listeria monocytogenes*

2.2.5.1. General Characteristics

Listeria spp. are Gram-positive, non-spore forming, facultatively anaerobic rod-shaped bacteria. Of the 17 species identified to date, *L. monocytogenes* remains the only member of this genus that is pathogenic to humans and animals. *L. ivanovii* is the other species that, although rare, has been shown to cause disease in ruminants (Orsi et al. 2011). There are four major *Listeria monocytogenes* serovars isolated from food and patients (1/2a, 1/2b, 1/2c, and 4b) and many outbreaks of invasive listeriosis are associated with serotype 4b strains. Premature stop codons in the gene *inlA* of 1/2a, 1/2b and 1/2 c strains may result in reduced infectivity of these serovars (Buchanan et al. 2017).

L. monocytogenes is a facultative intracellular pathogen. The organism is unusual in its ability to cross the intestinal, blood-brain and placental barriers (Doran et al. 2013). The majority (99%) of the infections caused by this pathogen are thought to be foodborne (Orsi et al. 2011). The pathogen is ubiquitous in nature and has been found to exist in many diverse environments including soil, water, vegetation, farm environments and food processing environments, sewage, and animal feed (Sauders et al. 2012; Ryser et al. 1997; Arimi et al. 1997).

Listeriosis is characterised by two primary syndromes, an invasive form of the illness versus a non-invasive form (Buchanan et al. 2017). Invasive illness is characterised by the onset of severe symptoms, including meningitis, septicaemia, primary bacteraemia, endocarditis, non-meningitic central nervous system infection, conjunctivitis, flu-like illness and spontaneous late-term abortions in pregnant women. Non-invasive illness results in febrile gastroenteritis. The median incubation period for invasive illness prior to onset of symptoms is approximately 30 days, versus 24 hours for the non-invasive form. Gastrointestinal symptoms are observed in approximately one-third of documented cases of listeriosis (Ooi and Lorber 2005). For the year 2014, there were 2,194 confirmed listeriosis cases in the EU and 210 deaths (an increasing trend), with 98.9% of those cases hospitalised³². Health Protection Scotland (HPS) reported 15 cases of listeriosis in Scotland in 2016³³.

General morbidity and mortality estimates of foodborne disease in the U.S. by the Centers for Disease Control and Prevention (CDC) indicate an incidence rate of 0.3 cases per 100,000 population³⁴, which compares to UK incidence of 0.29 cases per 100,000 population³⁵. While listeriosis is a relatively rare human illness, it remains a leading cause of death from a foodborne pathogen, with high mortality rates (20-30%), typically occurring among elderly or immunocompromised patients and pregnant women

³² <https://ecdc.europa.eu/en/publications-data/listeriosis-annual-epidemiological-report-2016-2014-data>

³³ <https://www.hps.scot.nhs.uk/giz/wrdetail.aspx?id=73166&wrttype=9>

³⁴ <https://www.cdc.gov/mmwr/volumes/67/wr/pdfs/mm6711a3-H.pdf>

³⁵ https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/712007/listeria_data_2006_to_2016_may_2018.pdf

2.2.5.2. *Listeria* spp. in raw milk

While raw milk may contain *L. monocytogenes*, the primary route of *Listeria* contamination of dairy products results from environmental contamination from the processing environment. Combined data from numerous surveys conducted worldwide suggests that approximately 2.2% to 3.8% of bulk tank raw cow's milk is likely to contain *L. monocytogenes* (Farber and Peterkin 1991b). When present, levels in raw milk are often very low (<1 to 1.0 cfu *Listeria*/ml) (Lovett et al. 1987; D'Amico et al. 2008) with sporadic contamination and seasonal variability.

Abou-Elainin et al. (2000) analysed 450 goats' milk samples obtained from the bulk tanks of 39 goat farms for *Listeria* spp. over a 1-year period. Overall, 35 (7.8%) samples yielded *Listeria* with *L. monocytogenes* identified in 3.8% of *Listeria*-positive samples, and *L. innocua* (an important indicator for *L. monocytogenes*³⁶) identified in 5.8% of samples. Eight milk samples contained both *L. monocytogenes* and *L. innocua*. Milk samples from 46.2% of farms were positive for *Listeria* at least once during the year-long study. Molecular subtyping revealed five different *Listeria* subtypes from 34 selected *L. monocytogenes* isolates, two of which were deemed to be of clinical importance, showing genetic relatedness to strains linked to human clinical cases from previous illness investigations. Isolation rates of *Listeria* were markedly higher during the winter (14.3%) and spring (10.4%) compared to autumn (5.3%) and summer (0.9%).

The SCA detected *L. monocytogenes* in 43 of 639 milk samples (6.7%) from UK cheese makers collected during January 2011 and August 2012. *L. monocytogenes* can persist in processing environments for 12 years or longer (Orsi et al. 2008) in the absence of interventions to eradicate the source of contamination. The SCA noted that during the survey, two cheesemakers experienced a *Listeria* contamination incident that required additional testing and resulted in an elevated number of isolates. Thus, the results presented are likely reflect a higher *Listeria* incidence that would be found normally during milk surveillance.

2.2.5.3. Fate of *L. monocytogenes* in Cheesemaking

L. monocytogenes contamination has been found in ready-to-eat (RTE) foods including raw milk, pasteurised milk, and processed meat and poultry, which have all caused outbreaks (Nightingale et al. 2004). If pathogens are present in raw milk, they can be present throughout a cheese. Environmental contamination could be restricted to the cheese surface, or may be distributed throughout a cheese depending whether contamination occurred to the milk, curds in a vat or a finished wheel in an ageing room. Most outbreak investigations have failed to pinpoint the exact route of contamination in outbreaks. This is mainly due to the fact that investigations are retrospective and not real-time and the environmental conditions that existed when the cheese may be very different than the environmental conditions when sampling is undertaken. Some

³⁶ *L. innocua* is non-pathogenic and not a food safety concern. FBOs should review conditions and parameters that may have permitted *L. innocua* to be present in milk (silage feeding/silage quality being the chief parameter).

cheeses age for many months and sources of contamination, such as shedding patterns of cattle and feed, may change throughout this time. Therefore, a HACCP or other food safety plan should address all potential routes of environmental contamination.

L. monocytogenes is tolerant to environmental stresses and can grow at temperatures between 0.4-45°C as well as A_w and pH values between 0.90-0.97 and 4.3-10.0, respectively (Farber and Peterkin 1991a). *L. monocytogenes* is also capable of growing in a range of salt concentrations (up to 10%) substantially higher than those found in cheese, and has been shown to survive for months in salt concentrations of up to 26% under refrigeration in broth studies (Ryser 2007). While salt levels in cheeses would not approach this level, the combination of salt, pH and A_w can interact to create an environment hostile to the growth of *Listeria*, as has been shown for Cheddar cheese (Ryser and Marth 1987).

Listeria is inactivated by pasteurisation and contamination of processed dairy products made from pasteurised milk is therefore most likely a function of post-pasteurisation contamination from the dairy plant environment. Results of quantitative risk assessments conducted in the U.S. and Europe identified RTE foods contaminated as the result of post-processing contamination as the cause of most cases of foodborne listeriosis (FDA 2003; WHO 2016).

The ability of *L. monocytogenes* to survive under stressful environmental conditions including high salt, low pH and cold temperatures make this pathogen not only very difficult to control in production, but also extremely persistent in the environment. Recently published studies have shown the contribution of molecular determinants to adaptation and persistence of *Listeria* strains, as well as resistance to sanitisers (Harter et al. 2017; Pan et al. 2006; Buchanan et al. 2017; Kremer et al. 2017). *L. monocytogenes* is widely distributed in dairy farm environments (Nightingale et al. 2004) and is regularly isolated from dairy processing and cheesemaking environments (Nightingale et al. 2004; Pritchard et al. 1994; D'Amico and Donnelly 2010).

Some strains of *L. monocytogenes*, including those that may possess increased virulence by virtue of their association with human clinical cases, have been shown to persist in cheesemaking (D'Amico et al. 2008; D'Amico and Donnelly 2009) and other food processing environments for months or years (Ferreira et al. 2014) and serve as sources of food product contamination. Effective environmental monitoring, a legal obligation in the Regulation (EC) 2073/2005 and elimination of *Listeria* spp. within processing plants, including farmstead cheese operations, is thus a key component of a *Listeria* control program. Risk reduction efforts should be placed on the identification of reservoirs of pathogens such as *Listeria* in the production system and the development of practices that reduce the spread of pathogens and, as a result, minimise the risk of cheese contamination.

Listeria's ubiquity is due to its ability to form biofilms and resist sanitisers, making removal extremely difficult (Pan, Breidt, and Kathariou 2006). In fact, numerous surveys

document the presence of *Listeria* spp. within the dairy plant environment including floors, drains, freezers, processing rooms (particularly entrances), cases and case washers, floor mats and foot baths (Pritchard et al. 1994; D'Amico, et al. 2008; D'Amico and Donnelly 2009). Pritchard et al. (1994), in a study of dairy processing facilities, found that processing plants near a farm had a significantly higher incidence of *Listeria* contamination than those without an on-site dairy farm. Arimi et al. (1997) demonstrated the link between on-farm sources of *Listeria* contamination (dairy cattle, raw milk and silage) and subsequent contamination of dairy processing environments. These investigators subjected *Listeria* strains collected from farms and dairy processing environments over a 10 year period to molecular subtyping. A total of 388 *Listeria* isolates from 20 different dairy processing facilities were examined along with 44 silage, 14 raw milk bulk tank and 29 dairy cattle isolates. The finding of eight *L. monocytogenes* and twelve non-*L. monocytogenes* subtypes common to both dairy processing and farm environments supports the farm as a natural reservoir for *Listeria* contamination of dairy processing facilities.

A study of Irish Farmhouse cheese processing environments supported similar conclusions regarding the farm as a reservoir for *Listeria* (Fox et al. 2011). These findings, which support the link between on-farm sources of *Listeria* contamination (dairy cattle, raw milk and silage) and subsequent contamination of dairy processing environments, stress the importance of farm-based programs for controlling *Listeria*. Controls must include regular environmental testing at the farm, to verify absence of plant environmental niches and contaminated surfaces that come into contact with cheese, and should also include regular bulk milk tank filter testing for milk used to make cheeses that can support the growth of *Listeria*.

All cheeses, whether made from pasteurised or unpasteurised milk, are at risk of containing *L. monocytogenes* due to post-processing contamination that can occur during manufacture, as well as during ripening and washing, or at retail (Jacquet et al. 1993; Gaulin et al. 2012). Routine environmental monitoring to verify the efficacy of cleaning and plant sanitation is essential, and is in fact a legal requirement under Regulation (EC) 2073/2005³⁷. Washed rind cheeses represent a class of high risk cheeses for which contamination with *L. monocytogenes* is well documented (Pichler et al. 2011). Many of these cheeses are traditional European varieties having Protected Designation of Origin (PDO) status. EU regulations governing the production of these cheeses allow the use of traditional tools and practices. Washed rind cheeses, which include such varieties as Limburger, Taleggio, Époisses and Munster, are washed with a brine or smear that promotes the development of a viscous, red-orange microbiological consortium composed of bacteria and yeasts. This surface growth causes the cheese pH to increase from approximately 5.0 to 7.0, which could enable the growth of *L. monocytogenes* to high levels if present on the cheese surface (Pichler et al. 2011; Rudolf and Scherer 2001).

³⁷ <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:338:0001:0026:EN:PDF>

The washing of cheeses with a brine solution represents a major route of contamination and cross-contamination with *L. monocytogenes* (Pichler et al. 2011; Carminati et al. 2000) and brine solutions must be properly maintained and monitored for presence of contaminants including *Listeria*. While scant information is available in the scientific literature regarding methods for control of brines used as cheese wash or smear solutions, guidance is offered³⁸ for maintenance of brines used for salting of cheese as follows:

- Control brine strength by monitoring salt records (50% saturation recommended);
- Control brine pH and temperature (pH of the brine should be equal to that of the cheese);
- Record details of batches and time spent in brine;
- Pasteurise brine;
- Replace at regular intervals;
- Regularly clean brine tanks;
- Filter/sieve brine (to remove any cheese particulates if the brine is being reused);
- Microfiltration/UV treatment;
- Chlorinate;
- Conduct microbiological testing

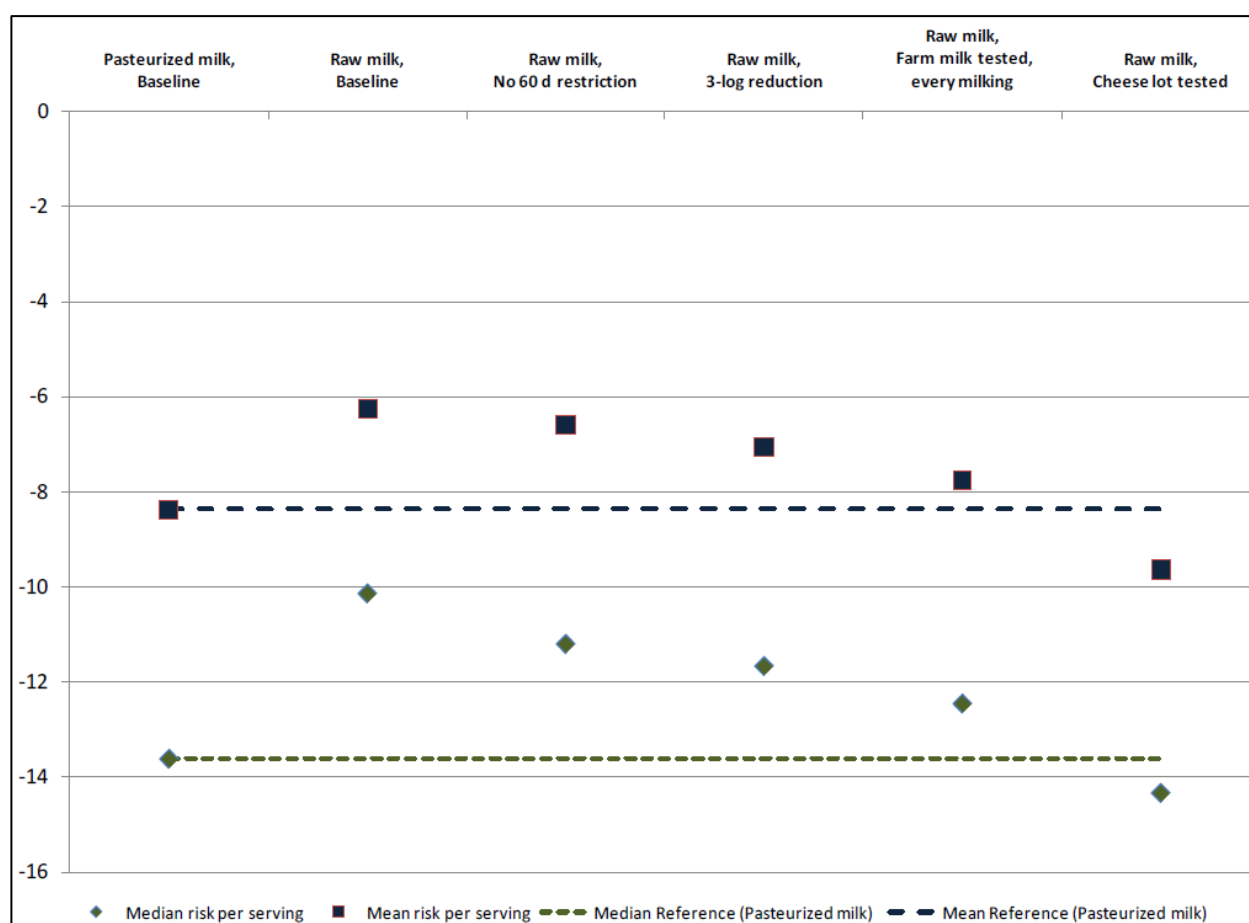
Work conducted by D'Amico and Donnelly (unpublished) identified “smear” or “wash” application devices such as brushes and sponges as a source of *Listeria* spp. dissemination across production units of washed rind cheeses at a commercial producer. The soaking of the applicator in sanitiser overnight proved ineffective for the complete elimination of *L. monocytogenes*. Unpublished work from the Donnelly laboratory has shown that boiling applicators such as brushes or sponges in water after use is a more reliable means of inactivating contaminants. The efficacy of disinfection strategies for the elimination of *L. monocytogenes* present on cheese washing materials has not been fully examined. With growth of the artisan cheese industry and increased consumer demand for washed rind cheese, these products could serve as further potential vehicles of foodborne illness.

In 2015, the U.S. FDA and Health Canada published results of a joint Soft Cheese Risk Assessment (FDA 2015). FDA and Health Canada have documented associations between consumption of certain soft cheeses and the onset of listeriosis and therefore they conducted the risk assessment to evaluate the safety of soft-ripened cheeses; particularly those made from raw milk. The public health impact of *L. monocytogenes* in soft ripened cheese was assessed through focus on sources of contamination, the impact of various manufacturing and processing steps, and the effectiveness of intervention strategies, including new technologies. The impact of consumer handling practices was also evaluated, and a model developed to assess predicted risk associated with manufacturing processes, interventions, and handling practices.

³⁸<https://dairyextension.foodscience.cornell.edu/sites/dairyextension.foodscience.cornell.edu/files/shared/Cornell%20Dairy%20Extension-Brine%20Maintenance%20SOP.pdf>

Conclusions from this risk assessment showed that testing every batch of soft ripened cheese made from unpasteurised milk for *L. monocytogenes* achieved a mean level of safety higher than untested cheese made from pasteurised milk. Although not a legal requirement, testing each lot of cheese (with a lot legally defined by Codex as “a definitive quantity of a commodity produced essentially under the same conditions” and as “a batch of sales units of food produced, manufactured or packaged under similar conditions” in the UK by the Food (Lot Marking) Regulations 1996³⁹) for *L. monocytogenes* is an example of an evidence-based risk management option available to cheesemakers to ensure cheese safety, evidence of this is shown in Figure 3 (for the elderly population only).

Figure 3: Log₁₀(median) (♦) and log₁₀(mean) (■) risk per serving at random for the Elderly population, Canada, comparing soft-ripened cheese made from pasteurised milk baseline, soft-ripened cheese made from raw milk baseline, farmstead raw-milk cheese without 60-day ageing regulation, farmstead raw-milk cheese with a 3-log reduction of *L. monocytogenes* concentration in milk, farmstead raw-milk cheese with milk testing, farmstead raw-milk cheese with cheese lot testing⁴⁰



³⁹ <http://www.legislation.gov.uk/uksi/1996/1502/regulation/2/made>

⁴⁰ <https://www.fda.gov/Food/FoodScienceResearch/RiskSafetyAssessment/ucm429410.htm>

Food Standards Australia New Zealand (FSANZ) received a request for French Roquefort cheese to be sold in Australia, and this request resulted in production of a risk assessment (FSANZ 2009⁴¹). In reviewing this request, FSANZ determined that the legal requirements of the French regulatory system (Ministerial Order of 1994) for raw milk and Roquefort cheese manufacture were considered comprehensive and adequate for safety assurance. Among the legal requirements found to reduce the risk was raw milk testing for *L. monocytogenes* of each milk tanker load for every batch of cheese produced. During cheese production, pH, salt concentration and moisture levels are monitored, and the minimum maturation period is no less than 90 days. Established microbiological limits mean that Roquefort cheese must have no detectable levels of *L. monocytogenes* and salmonella at retail.

Additionally, a qualitative risk assessment (FSANZ 2009³⁹) undertaken by Food Science Australia to categorise the risk from each potential pathogen in Roquefort cheese showed negligible to low risk for seven pathogens (including *Coxiella burnetti*, *Brucella melitensis* and *Campylobacter jejuni*). Based on the qualitative risk assessment, the sale of Roquefort is permitted in Australia. The risk assessment concluded that there is a very low/negligible risk of listeriosis if *L. monocytogenes* is not present in raw milk used for cheese manufacture and there is effective control over cheesemaking and ripening operations. *L. monocytogenes* is unlikely to grow in Roquefort cheese during maturation and subsequent storage due to low pH and A_w . Given the relatively low consumption rates of Roquefort in Australia, the risk assessment predicted three cases per year in immunocompromised individuals. An additional requirement in Australia is the labeling of Roquefort at retail “Made from unpasteurised ewe’s milk”, consistent with EU labeling requirements.

Bacteriophages have been successfully used for control of foodborne pathogens such as *L. monocytogenes* in cheeses as they inactivate target bacterial cells with inherent specificity and do not affect starter and ripening cultures (Carlton et al. 2005; Guenther and Loessner 2011). Listex™ P100 is a lytic phage characterised by its broad host range within the genus *Listeria*. EFSA confirmed the safety of Listex™ P100 in 2015, and the EU Commission approved its use in 2017. It is not, however, currently approved for use in the UK for ready to eat products of animal origin⁴². Its efficacy for control of *L. monocytogenes* as a surface contaminant on soft-ripened cheese was explored by Carlton et al. (2005) who found significant *L. monocytogenes* reductions (3.5 logs). Similar reductions were reported by Guenther and Loessner (2011) who found that efficacy of phage treatment varied by initial *Listeria* contamination levels. When cheeses received an initial inoculation of 1×10^3 cfu/cm² of *L. monocytogenes* strain Scott A (an outbreak-associated serotype 4b strain), application of phage resulted in a 3 log reduction after 22 days compared to control cheeses. When initial contamination was reduced (1×10^2 cfu/cm² and 1×10^1 cfu/cm²), differences in cell counts of more than 6 logs were achieved, and no viable cells could be recovered by direct plating after day 6.

⁴¹ http://www.foodstandards.gov.au/code/applications/Documents/A499_Roquefort_FAR_FINALv2.doc

⁴² https://ec.europa.eu/info/law/better-regulation/initiatives/ares-2017-3512172_en

Thus, initial killing efficacy of the phage and the final difference in viable cell count was significantly better when the initial *Listeria* concentration was low (1×10^2 cfu/ cm² or below). Carlton found that Listex™ P100 was sufficiently stable, with no decrease or increase in phage titers as determined over a period of 6 days. None of the *L. monocytogenes* clones isolated from cheeses demonstrated resistance against the phage.

In contrast, Guenther and Loessner (2011) found that although all 10 Scott A (a clinical strain) isolates remained fully sensitive to phage A511 infection, three out of ten (30%) clones of another strain, CNL 103/2005, recovered from 22 day old phage treated cheese samples showed a phage-insensitive phenotype. This may pose an issue with respect to washed rind soft cheese production and the traditional practice of “old-young smearing”, where the rind microflora from mature cheeses is used to wash the young cheeses (Guenther and Loessner 2011). Fister et al. (2016) investigated use of Listex™ P100 for environmental control of *Listeria* under conditions normally found in dairy plants and also observed development of phage-resistant strains of *L. monocytogenes*. When their use is permitted, cheesemakers considering the use of bacteriophages for *Listeria* control should be aware of the potential limitations (phage resistance) associated with this control strategy.

2.2.5.4. Outbreaks of *Listeria* associated with Cheese

Numerous outbreaks and sporadic cases of listeriosis have been linked to the consumption of soft fresh cheeses (Farber 1990; De Buyser et al. 2001; Linnan et al. 1988; Amato et al. 2017), as well as soft surface-ripened cheeses. Soft surface-ripened cheeses are soft cheeses that undergo further ripening through the external growth of yeasts, moulds and/or bacteria. Soft and semi-soft surface ripened cheeses and smear ripened or washed-rind cheeses include well-known varieties such as Camembert, Limburger and Taleggio.

Post-processing contamination of soft surface-ripened cheese is of critical concern as pathogen growth parallels the increasing pH during ripening (Ryser and Marth 1987; D'Amico et al. 2008). The increase in pH during cheese ripening can create a favorable environment which enables the growth of *L. monocytogenes* to high levels (Pichler et al. 2011; Ryser and Marth 1987). Additionally, if present, *L. monocytogenes* can survive and continue to grow during refrigerated storage of cheese due to its psychrotrophic nature, highlighting the need to prevent environmental contamination of soft ripened cheeses during production. Investigation of a recent U.S. outbreak of listeriosis linked to cheeses produced by the Vulto Creamery revealed widespread *Listeria* contamination throughout the cheesemaking facility, and the creamery is now permanently closed⁴³.

Perhaps the most well-known outbreak of listeriosis was linked to the consumption of the washed rind cheese Vacherin Mont d'Or, and occurred in Switzerland from 1983–1987, involving 122 cases. The cases were of uniform geographic distribution by patient

⁴³ <https://www.fda.gov/Food/RecallsOutbreaksEmergencies/Outbreaks/ucm545787.htm>

residence (with most patients (77%) hospitalised at a University-affiliated tertiary care center for the 500,000 residents of the canton of Vaud in Western Switzerland) and peaked during winter months. Investigators suspected a common source, but results of two case–control studies addressing a variety of food, occupational, and household exposures were inconclusive. In an independent effort, Swiss health officials conducted studies to determine the prevalence of *L. monocytogenes* in cheese and other dairy products. *L. monocytogenes* was isolated from regionally produced Vacherin Mont d'Or soft cheese, including the two predominant phage types (PTs) found in the patients. This high-risk product was not previously recognised as a vehicle of foodborne illness (Bula et al. 1995). Swiss officials tracing the source of contamination in this outbreak recovered the epidemic strain of *L. monocytogenes* from 6.8% of the wooden shelves and 19.8% of brushes used in the ripening cellars. Thus, brushing cheese with smear and ripening cheese on wooden shelves appeared to be two important means for dissemination of *L. monocytogenes* within cheesemaking facilities (Gurtler and Kornacki 2007).

Amato et al. (2017) identified a major listeriosis outbreak that occurred during 2009-2011 involving 43 cases in Northern Italy linked to Taleggio cheese: a semi-soft, washed-rind, smear-ripened Italian cheese. The outbreak went undetected until DNA-sequence based typing methods were integrated with traditional molecular subtyping methods (PFGE) to reveal a novel epidemic clone in a retrospective analysis of clinical isolates collected in Lombardy between 2006 and 2014.

In the U.S. FDA's Domestic and Imported Cheese Compliance program results from 2004 to 2006, 42 of 2,181 (1.9%) of imported cheese samples tested positive for *L. monocytogenes*, compared to domestic cheese samples where 10 of 2181 (0.45%) were positive. The EFSA survey of presence of *L. monocytogenes* in cheese samples from EU Member States showed that the incidence of the pathogen was 0.47% for the time period 2010 to 2011 (European Food Safety 2013). The incidence of *L. monocytogenes* reported by EFSA agrees with the findings of (Lambertz et al. 2012) where the authors reported that in cheese samples from Sweden, the incidence of the pathogen was 0.4%.

The risk of cheese cross-contamination at retail when cheeses are cut and wrapped or sliced has been addressed in recent publications (Little et al. 2008; Sauders and D'Amico 2016) . Such contamination complicates trace back investigations to identify the source of contamination in illness outbreaks. These authors stressed the need for application and maintenance of good hygienic practices throughout the food chain in order to prevent contamination and minimise growth. As an example, an imported Ricotta Salata (a soft cheese made from pasteurised milk), was identified as the causative agent of a complicated outbreak of listeriosis that occurred in the U.S. in 2012. The outbreak affected 22 individuals in 13 states (Heiman et al. 2016). Investigations began in Pennsylvania where a patient who had contracted listeriosis had consumed two soft cheeses purchased from a grocery store: a commercially produced

blue cheese made from unpasteurised milk and an imported l'Édel de Cléron made from pasteurised milk. Investigators postulated early in the investigation that an intact contaminated cheese could cross-contaminate multiple cheese types during cutting and wrapping. The outbreak strain was isolated from samples of cut and repackaged cheese from both a cheese distributor and a grocery chain. The distributor did not ship, cut and repackage cheese to the grocery chain, and the grocery chain received only intact wheels. Epidemiological investigations revealed that blue and farmstead cheeses that were cut and repackaged by the distributor were contaminated with the epidemic strain of *L. monocytogenes*, but intact wheels of blue and farmstead cheese did not contain *L. monocytogenes*. Cutting records at the distributor revealed that Riccota Salata was the only common cheese used at cutting stations for the blue and farmstead cheese. At the grocery store, it was likely that Riccota Salata likely cross-contaminated the blue cheese and l'Édel de Cléron bought by the Pennsylvania patient. The outbreak illustrates the risks of cross-contamination posed by contaminated cheese, and illustrates the need for use of validated disinfection protocols and sanitation of wire cutters, cutting boards, knives and utensils following cutting and wrapping of cheese blocks.

2.2.6. *Staphylococcus aureus*

2.2.6.1. General Characteristics

Coagulase positive staphylococci (CPS) including *Staphylococcus aureus*, *S. intermedius* and certain strains of *S. hyicus* are of concern to cheesemakers due to their production of thermo-stable enterotoxins that cause foodborne illness. Of these species, *S. aureus* remains one of the most important and costly pathogens for the dairy industry. Enterotoxigenic strains, including *S. aureus* strains can induce foodborne intoxications through dairy products including cheeses (Le Loir et al. 2003; Cretenet et al. 2011). Although two coagulase-positive species (*S. hyicus* and *S. intermedius*), and 10 coagulase-negative species contain toxigenic strains, most reported cases of staphylococcal food poisoning are linked to *S. aureus* (Ryser 2012).

Once ingested, staphylococcal enterotoxins (SE) act on emetic receptors in the intestinal wall producing nausea, vomiting, diarrhoea and abdominal cramps within 1 to 6 hours following ingestion of the contaminated food. Recovery generally takes one to two days and rarely results in complications (that would be mainly due to dehydration) or hospitalisation (Ryser 2012; Cretenet, et al. 2011). In addition to the classical staphylococcal enterotoxin types (SEA, SEB, SEC, SED and SEE), extensive sequence data have led to the discovery of novel SEs and staphylococcal enterotoxin-like super-antigens whose potential role in staphylococcal food poisoning (SFP) in many cases have yet to be confirmed (Lina et al. 2004). It is assumed that SFP outbreaks are under-reported due to symptoms that are less severe than those associated with other microbial pathogens. SE type A causes the majority of staphylococcal illness worldwide (Kadariya et al 2014).

S. aureus is a major causative agent of mastitis and one of the most common contagious pathogens infecting dairy cows. The pathogen is regularly isolated from the raw milk of domestic milking species. Average incidence rates for raw cows' milk are in the range of 20-30%, while the incidence for goat and ewes' milk is typically between 30 and 40% (Cretenet, et al. 2011; De Reu et al. 2004; D'Amico et al. 2008; Tham et al. 1990). Some surveys, however, report prevalence rates as high as 75% and 96% for cow and goats' milks, respectively (Kousta et al. 2010; Jørgensen et al. 2005). Between 30-50% of the human population asymptotically carries *S. aureus* in their nostrils, skin and hair. Milk and milk products can become contaminated prior to or following heat treatment during processing and human handling (Le Loir et al. 2003).

Results of a study by Tondo et al. (2000) suggest that personnel may not play a major role in the contamination of dairy products with *S. aureus*; especially when compared to contamination of the raw milk itself. Equipment and machinery are not identified as potential sources of contamination, but it is recognised that *S. aureus* strains can form biofilms that may play a role in on-farm persistence (Thiran et al. 2017). Most outbreaks linked to the use of raw milk have been traced to mastitic dairy cows whereas contamination of processed products occurs post-pasteurisation through improper handling and human transmission (Ryser 2001). The proportion of dairy related

illnesses from staphylococcal poisoning in the U.S. has decreased substantially in the past 40 years as a result of increased monitoring of mastitis in dairy cattle coupled with improved sanitation and the implementation of pasteurisation (Ryser 2001). Similar trends have been observed in the UK⁴⁴. However, despite similar improvements, *S. aureus* has been reported as the leading cause of foodborne disease related to milk and milk products in France (De Buyser et al. 2001) possibly resulting from the use of raw milk.

When grown at temperatures above 7°C, *S. aureus* displays acid and salt tolerance with demonstrated growth in acidic environments as low as pH 4.0 and salt concentrations as high as 25% (D'Amico and Donnelly 2017). *S. aureus* has also been shown to grow in laboratory media at water activity (A_w) levels as low as 0.83-0.86 (Genigeorgis 1989).

2.2.6.2. Fate of *S. aureus* in Cheesemaking

In addition to *S. aureus* contamination of raw milk (Bone et al. 1989; Kousta et al. 2010), outbreaks and recalls of cheese manufactured from pasteurised milk occur from staphylococcal enterotoxin (SE) production in milk prior to heat treatment, or as a result of post-pasteurisation contamination (Altekruse et al. 1998; Le Loir et al. 2003; Cretenet et al. 2011). With use of active lactic acid starter cultures that assure rapid acidification during cheesemaking, *S. aureus* is considered to be a low risk pathogen because it is generally recognised as a poor competitor with other bacteria, particularly lactic acid bacteria (Johnson et al. 1990). However, in traditional cheeses where active starter cultures are not utilised, *S. aureus* may pose a significant risk for toxin production in cheese if numbers are sufficiently high (Zárate et al. 1997). The inhibitory effect of lactic starter cultures is related to, and dependent upon, the ratio of starter organisms to pathogen, the amount and type of starter culture added, competition for nutrients, decreasing pH as well as the production of hydrogen peroxide (H_2O_2), undissociated weak acids and inhibitory metabolites (Genigeorgis 1989; Charlier et al. 2009). Reducing the risk associated with toxin production in cheese of all varieties is dependent upon assuring low levels of *S. aureus* in both raw and pasteurised milk (Delbes et al. 2006; Cremonesi et al. 2007). The SCA found that 91% of UK raw cows' milk samples examined from January 2011 to August 2012 (Table 6) had undetectable coagulase positive staphylococci (<20/ml) and only 4% had levels exceeding 100/ml (SCA Technical Committee, October 2012, personal communication).

⁴⁴ https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/638891/hpr2917_stph-aurs.pdf

Table 6: Coagulase-positive staphylococci results from SCA survey (2102) of microbiological quality of raw milk from different species (Jan 2011-Aug 2012). Data from SCA Technical Committee, October 2012

Milk type	No. Tests	<20 cfu/ml		20–100 cfu/ml		100-1000 cfu/ml		≥1000/ml		Highest Count (cfu/ml)
		No. samples	%	No. samples	%	No. samples	%	No. samples	%	
Cow	548	501	91%	21	4%	24	4%	2	0%	1,120
Sheep	23	9	39%	0	0%	13	57%	1	4%	3,720
Goat	27	24	89%	0	0%	3	11%	0	0%	300

EU microbiological criteria (EC 2073/2005) require cheesemakers to test for the presence of *S. aureus* at the point of cheese production when counts are expected to be the highest; the microbiological criteria also informs cheesemakers of the limit over which enterotoxin testing will also have to be performed. While EU microbiological criteria require raw milk cheese to have *S. aureus* levels of <10,000 cfu/g, the SCA recommends that cheese producers aim for <100 cfu/g. The SCA data (Table 6) indicates that this standard is easily attainable as evidenced by only 43/598 tested samples (7.2%) exceeding 100 coagulase positive staphylococci/ml. Cheeses must be tested for staphylococcal enterotoxins (SEs) if coagulase-positive staphylococci are detected at levels >10⁵ cfu/g.

Cretenet et al (2011) reported *S. aureus* to be an important pathogen in soft and semi-soft cheeses, particularly in cheeses where starter cultures are not used. Growth occurs primarily in the first phase of cheesemaking from inoculation to salting. Fermentation processes reaching high levels of LAB become inhibitory to *S. aureus* and inhibit enterotoxin formation. Enterotoxins may be produced before the pH drops to inhibitory levels if initial levels of *S. aureus* present in milk are high (10⁴-10⁵ cfu/ml). Growth occurs in semi-hard and hard cheeses if the initial population in milk is high (10³ cfu/ml) and enterotoxins may be produced.

D'Amico and Donnelly (2011) characterised *S. aureus* isolates obtained from raw milk used for the production of artisan cheese in order to examine the genetic and phenotypic diversity, the enterotoxigenicity and the antimicrobial resistance. 90 isolates from cow, goat and ewes' milk collected during routine surveillance over a 3-year period were examined. Additional isolates collected from whey, brine, curd and human nasal samples were also analysed. 16 different subtypes were identified among the 90 food isolates examined that were typically associated with a specific animal species, with more than half of isolates unique to individual farms. Limited antimicrobial resistance was observed among the isolates, with resistance to ampicillin (15%) or penicillin (12%) as the most common. Two isolates of the same subtype obtained from the same farm

were resistant to oxacillin, an antibiotic used to treat mastitis, made up in 2% NaCl solution. In general, staphylococcal enterotoxin (SE) production, or the lack thereof, was also linked to specific subtypes and more than half (56%) of isolates produced toxin. Overall, 34 of the 38 isolates tested produced only toxin type C (SEC). The recurrence of individual subtypes on specific farms over time further illustrates the chronic nature of infection. Although these data demonstrate that strains found in raw milk intended for artisan cheese manufacture are capable of enterotoxin production, SEC is not typically linked to foodborne illness (Kadariya et al. 2014).

The unexpected finding of limited antimicrobial resistance (AMR) is an area that requires further investigation. In contrast, a 2010 study conducted on 28 artisanal farmhouse cheeses manufactured in Scotland found widespread resistance to methicillin and oxacillin among 25 *S. aureus* isolates recovered from cheeses (Williams and Withers 2010). This was the first study demonstrating the presence of methicillin resistant *S. aureus* in Scottish dairy products. However, unlike clinical strains of MRSA, the methicillin resistant cheese isolates displayed sensitivity to chloramphenicol, erythromycin, gentamycin and tobramycin. *S. aureus* was found in 40% of raw milk cheese varieties examined, with varieties made from organic milk (58% positive) showing a higher incidence than those made from non-organic milk (15% positive). Levels ranged from 10^2 to 10^5 cfu/g, with 50% of cheese samples exceeding the 10^4 *S. aureus* cfu/g limit established by Regulation (EC) 2073/2005. Seven of the 25 isolated *S. aureus* strains were able to form SEC. None of the 28 cheeses tested positive for *E. coli* O157:H7 or salmonella.

2.2.6.3. Outbreaks of *Staphylococcus aureus* associated with Cheese

Bone et al. (1989) reported on an outbreak of staphylococcal illness in Scotland from ewes' milk cheese made from unpasteurised milk. This outbreak gave rise to calls for mandatory pasteurisation of milk from goats and sheep for use in the production of milk products. Mandatory pasteurisation of cows' milk was shown to significantly reduce the risk of salmonellosis and campylobacteriosis in Scotland. Changes suggested to prevent future contamination included reliable and rapid refrigeration of milk following collection, more rapid warming of milk to fermentation temperature, and use of a commercial starter culture. Cheese pH from core samples of cheese involved in the outbreak ranged from 5.88 to 6.86. Following use of new commercial starter, pH reduction from 6.20 to 5.53 (average of the core samples) was accomplished, with concomitant reduction in the numbers and frequency of staphylococci in mature cheese samples. The authors suggested that cheesemakers should employ monitoring of pH during cheese production to ensure successful fermentation and rapid pH decrease.

Maguire et al. (1991) reported on an outbreak of food poisoning associated with Stilton cheese made from unpasteurised milk, but the aetiological agent, although suspected to be *Staphylococcus* enterotoxin based on the symptoms of the affected individuals and the reported incubation periods prior to onset of illness, was never identified despite extensive testing of the implicated cheese for the bacteria and its toxin.

2.3. Cheesemaking and Process Control

2.3.1. Control of Pathogens in Raw Milk

The results of numerous investigations reveal that improving milk hygiene is the most significant factor leading to the microbiological safety of cheeses made from raw milk (FSANZ 2009b⁴⁵; FSIA 2015; Jaakkonen et al. 2017; Doyle et al. 2017; Farrokh et al. 2013). Substantial microbial diversity is present in raw milk, and a single raw milk sample may contain 36 dominant microbial species (Montel et al. 2014). Milk microbial diversity is influenced by the overall farm management system, which varies from farm to farm. The teat surface serves as the main source of bacteria that are useful in cheese making (Irlinger et al. 2015; Verdier-Metz et al. 2012; Quigley et al. 2013). Risk mitigation strategies recommended by FSANZ for milk production include ensuring collection of milk from healthy animals that can be individually identified; use of milk hygiene controls to minimise contamination during milking, cooling, storage and transport, and using time and temperature controls during milk handling, storage and transportation (FSANZ 2009b). Certain raw milk cheese production may not include a process to achieve reliable inactivation of pathogens, so monitoring the microbiological quality of raw milk becomes critical. Additional risk factors include temperature control of raw milk, the acidification process, curd cooking, maturation/ripening, salt concentration, water activity, pH and addition of nitrate (FSANZ 2009⁴⁶).

In the U.S., Federal regulations⁴⁷ do not regulate the presence of pathogens in raw milk used for the manufacture of raw milk products, only the presence of pathogens in cheese. The requirements of these regulations is absence; if a pathogen is detected the cheese is considered adulterated. In a study completed in 2006, the overall milk quality and prevalence of four target pathogens in raw milk destined specifically for artisan cheesemaking was evaluated (D'Amico et al. 2008). Raw milk samples were collected weekly (June-September) from 11 Vermont farmstead cheese operations manufacturing raw milk cheese from bovine (5), caprine (4), and ovine (2) milks. Overall quality was determined through standard plate count (SPC) - equivalent to aerobic colony count (ACC) and total coliform counts (CC), as well as somatic cell counts (SCC). Additionally, samples were screened for *L. monocytogenes*, *S. aureus*, *Salmonella* spp., and *E. coli* O157:H7. For quantitative detection, raw milk was directly plated on chromogenic agar media. Overall, 96.8% of samples had SPC <100,000 cfu/ml, 42.7 % of which were <1000 cfu/ml. Although no U.S. federal standards exist for coliform levels in raw milk, 61% of samples tested were within pasteurised milk standards under the U.S. Pasteurised Milk Ordinance (PMO) at <10 cfu/ml, and 84.3% of samples contained <100 coliforms/ml. All bovine milk samples were within the limits of the PMO for SCC (<750,000/ml), and 88% met the stricter EU regulations of 400,000 cfu/ml. Furthermore,

⁴⁵<https://www.foodstandards.gov.au/code/proposals/documents/p1007%20ppps%20for%20raw%20milk%201ar%20sd1%20cow%20milk%20risk%20assessment.pdf>

⁴⁶<https://www.foodstandards.gov.au/code/proposals/documents/P1007%20PPPS%20for%20raw%20milk%201AR%20SD3%20Cheese%20Risk%20Assessment.pdf>

⁴⁷<https://www.fda.gov/downloads/food/guidanceregulation/guidancedocumentsregulatoryinformation/milk/ucm513508.pdf>

98.5% of all small ruminant samples were in compliance with U.S. PMO standards for caprine milk (SCC <1,000,000/ml). Of the 11 farms, 8 (73%) were positive for *S. aureus*, detected in 35% (46/133) of samples at an average level of 25 cfu/ml. *L. monocytogenes* was isolated from 2.26% (3/133) of samples (all bovine), two of which were from the same farm. *E. coli* O157:H7 was recovered from 1 sample (0.75%) of caprine milk. *Salmonella* spp. were not recovered from any samples (0/133).

Follow-on studies by D'Amico and Donnelly (2010) investigated the presence of four pathogens including *L. monocytogenes* in small-scale artisan cheese production facilities. Results indicate that milk intended for artisan cheesemaking can be of high microbiological quality, with a low incidence of pathogens (no *L. monocytogenes*, *E. coli* O157:H7 or salmonella was detected in 101 tested milk samples). Their research indicate the need for continuous microbiological monitoring of milk, cheese, and the production environment to ensure that the final product is safe for consumption. In addition, this study suggested that factors that are found in association with most small-scale producers including pasture feeding, seasonal milking, lack of extending milk holding and small herd sizes contribute positively to milk quality. Previous research was focused on preventing the growth and eliminating pathogens such as *L. monocytogenes* during production through implementation of safety protocols via HACCP. The results of this study showed that identification of farm niches where pathogens can survive can be valuable for small scale producers when they are creating their HACCP plans and can lead to overall greater farm hygiene, which in turn can lead to safer products.

2.3.2. Testing of Raw Milk and Milk Filters

Between June 2012 and June 2013, The Food Safety Authority of Ireland conducted a year-long study to establish the prevalence of pathogens including *L. monocytogenes*, *Campylobacter*, STEC, and salmonella in raw milk and/or raw milk filters from 211 Irish dairy farms producing milk from cows, sheep and goats (FSAI 2015). Hygiene indicators including generic *E. coli* and coagulase positive staphylococci were also monitored in raw milk (but not milk filters). Generic *E. coli* was enumerated using ISO 16649-2 (2001)⁴⁸, a pour plate method using Tryptone Bile X-glucuronide Agar (TBX). *E. coli* O26 and O157 were detected using ISO 16654⁴⁹. Of the 600 milk samples collected, 94% represented cows' milk, 5% goats' milk and 1% sheeps' milk. As 81% of the dairy farms supplied large-scale milk processors, application of these findings to artisan cheese production may not be relevant. Results of STEC testing were reported for milk filters, with 12 of 190 filters tested showing positive results for STEC-isolates of *E. coli* O157:H7 and O26 which had at least one *stx* gene detected. Corresponding information for raw milk is not available as raw milk was not tested for STEC. One *E. coli* O157:H7 isolate was detected which was deficient for *Stx*₁ and *Stx*₂ and *eaeA*, the gene associated with attaching and effacing lesion of enterocytes and *hlyA*, the plasmid located enterohaemolysin-encoding gene. In the same milk filter, an *E. coli* O26 strain

⁴⁸ <https://www.iso.org/standard/29824.html>

⁴⁹ <https://www.iso.org/standard/29821.html>

with *stx*₁ and *stx*₂ and *eaeA* and *hlyA* was isolated. Of 210 raw milk samples tested for *E. coli*, 94% had <100 cfu/ml and 66% <10 cfu/ml. The study authors found no correlation between *E. coli* numbers and presence of pathogens in raw milk samples.

The FSAI report also outlined the main sources of contamination of raw milk which include: the udder of an infected lactating animal; the external surface of the udder which becomes contaminated from animal faeces, bedding or mud; silage; human handling; improperly maintained or sanitised milking equipment including pipes, pumps and vats; contaminated water; and contaminated air entering the milking plant (clawpiece air bleeds). The authors also identified challenges associated with raw milk testing. Some of the limitations include: the sporadic nature of contamination; pathogens existing at low levels; the uneven distribution of pathogens in milk; numbers below detectable limits even though these levels may cause disease; lack of sensitivity of some detection procedures; and environmental conditions (FSAI 2015). The authors reported higher isolation rates for pathogens from in-line raw milk filters compared to raw bulk tank milk samples. 7% (13/190) of raw milk filter samples tested positive for either *E. coli* O157 and O26, and 12/13 of these samples had at least one Shiga toxin gene (*Stx*₁ or *Stx*₂) detected. The authors cautioned that the presence of pathogens on in-line milk filters does not always correlate with the presence of pathogens in bulk tank raw milk samples, but rather indicates the potential for milk to be contaminated.

Jaakkonen et al. (2017) reported on an outbreak of sorbitol-fermenting (SF) *E. coli* O157 linked to consumption of unpasteurised milk and farm visits in Finland. Since its first identification in Germany in 1988, SF *E. coli* O157 has emerged as an important cause of outbreaks and sporadic infections in Europe. The authors confirmed a cattle reservoir and transmission of SF *E. coli* O157 via unpasteurised milk, with eight culture-confirmed STEC infections. Six of the eight culture-confirmed cases were children, all of whom were hospitalised. Inspections of the implicated farm revealed deficiencies with milk hygiene, animal husbandry practices, poor farm hygiene, insufficient washing of udder cloths and excessive animal density. Several practices were observed that posed a risk for manure contamination of bulk tank milk. Despite this, the somatic cell counts and total bacterial counts of the milk remained good (<250,000/ml SCC; <50,000 total bacterial count; generic *E. coli* was not measured), questioning the value of these tests for assessment of milk contamination by pathogens such as STEC.

Farrokh et al. (2013) reviewed intervention strategies for preventing STEC contamination of milk and milk products. The primary defense is milking hygiene and prevention of faecal contamination of milk. Where raw milk is destined for raw milk cheese production, selection of farms and specific skills of producers are recommended. The authors also advocated for preservation of the natural microbial population of raw milk. As there is no singular processing intervention other than pasteurisation that would target STEC elimination, the authors advocated GHP and application of HACCP principles for risk reduction. Effective sanitation in dairy facilities, accomplished through use of alkaline cleaners and hypochlorite rinse solutions, have

been shown to inactivate STEC biofilms (Sharma et al. 2005), which have been shown to play a role in STEC persistence in dairy environments (Vogeleer et al. 2014; Vogeleer et al. 2016). A combination of hurdles at the farm level showed that dry bedding and maintaining animals in the same groups were identified as the most important measures. The occurrence of STEC in milk is low, therefore the authors concluded that end-product microbiological analysis for STEC would be unlikely to deliver meaningful reductions in associated risk for the consumer, nor would routine monitoring reduce the occurrence of associated cases. The authors suggested that microbiological criteria based on process hygiene such as *E. coli* or *Enterobacteriaceae* (EB), may prove useful as a validation, monitoring or verification tool for control measures. As concluded by the authors “the control of STEC in dairy products can only be accomplished by a set of measures across the entire cheese production chain, although the optimal combination of measures has yet to be determined.”

Doyle et al. (2017) used sequence-based microbiota analysis to identify possible sources of contamination of raw milk. Results highlighted the influence of the environment and farm management practices on the raw milk microbiota. Using sequencing, the authors found the teat surface as the most prevalent source of milk contamination, with herd faeces being the next most prevalent source of contamination. Considerable differences were found between individual milk samples versus bulk tank milk samples, perhaps due to bulk tank milk samples acquiring flora from milking machines and piping. The authors assessed the impact of teat preparation on milk and teat microbiota composition. *Lactococcus*, *Lactobacillus* and *Pseudomonas* were more prevalent in outdoor, non-teat prepped samples, which suggests that the application of teat prep significantly reduced numbers of these microbes in raw milk.

Advising artisan cheese producers making raw milk cheeses to eliminate silage feeding in favour of dry hay or pasture feeding is a strategy that shows promise to reduce potential for presence of *Listeria monocytogenes*, and potentially other pathogens, in milk used for cheesemaking. An article authored by Driehuis (2013) reviews other microbiological hazards which can be transmitted through silage feeding to milk used for artisan cheese production. Many Protected Designation of Origin (PDO) and Appellation d’Origine Contrôlée (AOC) European cheese varieties prohibit silage feeding for certain varieties of cheese due to known microbiological hazards associated with this practice. Callaway et al. (2009) reviewed the impact of feed on shedding of *E. coli*, noting that reductions in *E. coli* O157:H7 in food-producing animals prior to entering the food chain have great potential to reduce human illnesses. Distillers’ grains have been shown to increase the shedding of O157:H7 by cattle (Jacob et al. 2008; Synge et al. 2003; Dewell 2005). Variability was postulated to be due to intermediate end products in yeast fermentation. Previous studies have shown the impact of diet on *E. coli* O157:H7 populations in the gut. When cattle are provided high grain rations, starch escapes digestion by ruminal flora, allowing passage to the hindgut where starch undergoes fermentation. Abruptly switching cattle from high grain to all hay diets resulted in a 1,000-fold reduction of *E. coli* shedding within 5 days, and also reduced the ability of

surviving *E. coli* to survive acid shock (Diez-Gonzalez et al. 1998). Feeding distillers' grains can increase faecal shedding of *E. coli* O157:H7 due to decreased volatile fatty acid (VFA) concentrations. Ruminal and intestinal VFA concentrations limit *E. coli* populations due to their toxicity. Synge et al. (2003) found an increase in shedding of *E. coli* O157:H7 in cattle fed distiller's grains, with Dewell (2005) reporting a 6-fold increase in the odds of *E. coli* O157:H7 shedding following distillers' grain feeding.

Lekkas and Donnelly (Lekkas 2016) worked with select Vermont farms producing milk for artisan cheese production to understand practices that enhanced or decreased incidence of *Listeria* within the farm environment through a project entitled "Farm sources of *Listeria monocytogenes* and impact on the microbiological quality of milk destined for artisan cheese manufacture".

Table 7 depicts recent data from their ongoing study (Lekkas and Donnelly, unpublished). They compared *Listeria* incidence on four farms; two (Farms A and D) fed dry hay or fed cows on pasture, while two others (Farms B and C) fed silage to animals. In both Farms B and C, the same subtypes of *L. monocytogenes* found in silage were found in other areas of the farm environment, particularly in water sources. Lekkas (2016) were unable to detect the presence of *Listeria* in bulk tank milk from studied farms. Testing milk filters for presence of *Listeria* was more effective in identifying potential presence of *Listeria* in milk. *Listeria* is occasionally detected in raw milk used for artisan cheese production, and when present (i.e. detected in the milk filter but absent in the bulk milk), it is usually at levels below detection limits. Testing milk filters increases the sensitivity of detection and provides confidence in negative results.

Table 7: *L. monocytogenes* reservoirs on farms producing raw milk for cheesemaking

<i>Listeria monocytogenes</i> locations per farm and serotype identification per farm					
Farm	No. of isolates	Location	Subtype	Lineage	Serotype
A	3	Drain in bulk room	1042B	I	4b
		Personnel shoe	1045C	II	
		Entrance to bulk room	1039	II	1/2a
B	5	Lane holding area	1054	II	
		Water sediment	1039C	II	1/2a
		Milk filter	1039C	II	1/2a
		Entrance to bulk room	1030A	II	
		Silage	1039C	II	1/2a
C	5	Silage	1061	III	4a
		Bedding (Sand)	1061	III	4a
		Head rail	1061	III	4a
		Water bowl	1061	III	4a
		Side rail	1061	III	4a
D	3	Water bowl	1045B	II	1/2a
		Water pipe supply	1044A	I	4b
		Water	1062D	II	1/2a

2.3.3. Raw Milk Microbiological Criteria for Cheesemaking

Commission Regulation (EC) No 853/2004 sets criteria for raw milk production and cheesemaking. For raw cows' milk, the rolling geometric average (over two months, consisting of two samples/month) for plate count at 30°C is <100,000 cfu/ml, for cows' milk and the rolling geometric average over three months with at least one sample per month for somatic cell count (SCC) is <400,000 cfu/ml. For raw milk from other species, the required plate count is <1,500,000 cfu/ml (rolling geometric average over a two month period, with at least two samples per month).

Raw cows' milk used to prepare dairy products must have a plate count at 30°C of <300,000 cfu/ml immediately before processing. Raw milk from other species used to prepare dairy products (using a process that will not involve any heat treatment) must have a plate count at 30°C of <500,000 cfu/ml (rolling geometric average over a two month period, with at least two samples per month).

The SCA's Assured Code of Practice (SCA 2015) recommends more stringent microbiological criteria including coliform testing (<100 cfu/ml), plate count at 30°C of <10,000 cfu/ml and *S. aureus* counts of <100 cfu/ml for raw cows' milk (Table 8). The

SCA ACoP is consistent with guidance from FSANZ⁵⁰, calling for *S. aureus* in raw milk at levels of <100 cfu/ml, total plate count of <25,000 cfu/ml and *E. coli* at <100 cfu/ml, with absence of both salmonella and *Listeria monocytogenes* per 25 ml of tested raw milk. Figure 4 below summarises this information above and from section 2.3.1 in a flow diagram.

Table 8: Microbiological and compositional criteria for milk in the European Union (as per APPENDIX 5.2.1 SCA ACOP)

Milk	Test	Criteria in EU legislation	SCA Recommendations
All raw milk	Antibiotics and other contaminant residues	Must not exceed maximum residue levels	A PASS antibiotic test result
	Coliforms	Not specified in EU legislation	< 100 cfu/ml
	<i>Staphylococcus aureus</i>	Not specified in EU legislation	< 100 cfu/ml
	Non-toxigenic <i>Escherichia coli</i>	Not specified in EU legislation	< 100 cfu/ml
	Bactoscan count	Not specified in EU legislation	< 12(000) cfu/ml
Raw cows' milk	Plate count at 30°C	≤ 100,000 cfu/ml ⁵¹	< 10,000 cfu/ml
	Somatic cell count	≤ 400,000 cfu/ml ⁵²	< 250,000 cfu/ml
Raw cows' milk for preparation of dairy products	Plate count at 30°C	< 300,000 cfu/ml ⁵³	< 10,000 cfu/ml
Raw goats'/ewes'/buffaloes' milk not destined for heat treatment	Plate count at 30°C	≤ 500,000 cfu/ml ⁵⁴	< 10,000 cfu/ml

⁵⁰ <https://www.foodstandards.gov.au/code/proposals/Documents/P1022-Raw-milk-prods-AppR-SD1.pdf>

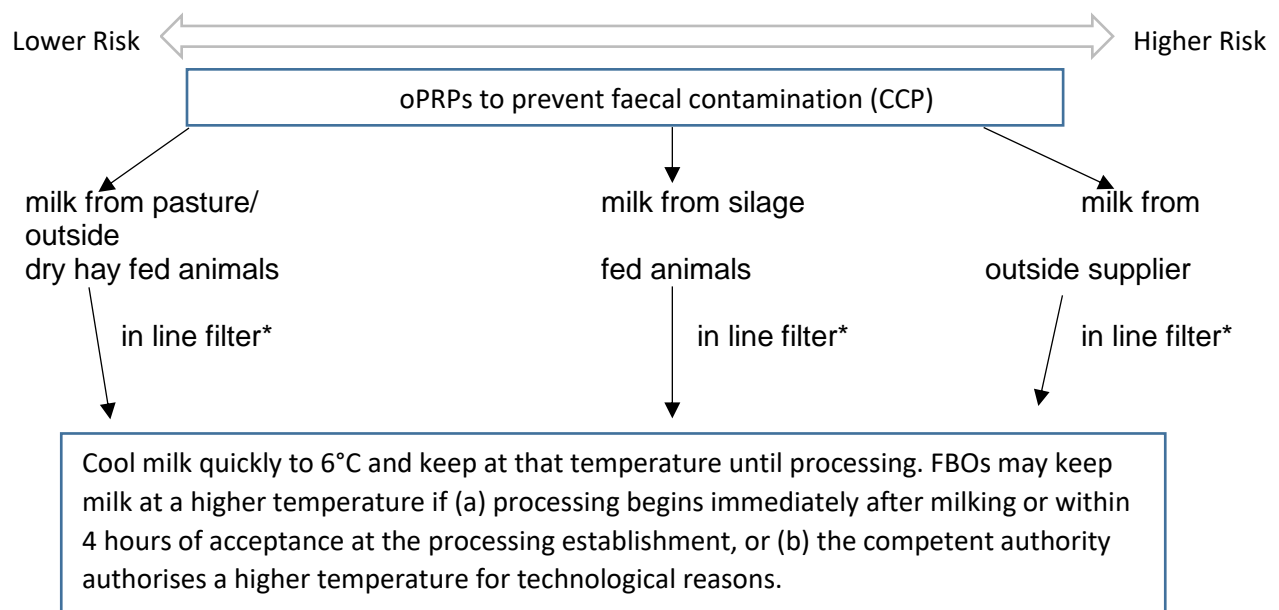
⁵¹ Rolling geometric average over two-month period, with at least two samples per month

⁵² Rolling geometric average over a three-month period, with at least one sample per month, unless the competent authority specifies another methodology to take account of seasonal variations in production levels

⁵³ Food business operators (FBOs) manufacturing dairy products must initiate procedures to ensure that, immediately before being heat treated and if its period of acceptance specified in the HACCP-based procedures is exceeded: a) raw cows' milk used to prepare dairy products has a plate count at 30°C of less than 300,000 per ml; and b) heat treated cows' milk used to prepare dairy products has a plate count at 30°C of less than 100,000 per ml. When milk fails to meet the criteria laid down in paragraph 1, the FBO must inform the competent authority and take measures to correct the situation (Regulation (EC) 853/2004).

⁵⁴ Rolling geometric average over two-month period, with at least two samples per month

Figure 4. Hazard Controls by Risk Level for Raw Milk for Cheesemaking



*may be tested for pathogens if cheese tests positive or following high generic *E. coli* or EB levels

Table 9: Factors for Managing Microbiological Risks of Unpasteurised Milk Production

Key Factor	What it facilitates	Evidence
oPRPs (personal hygiene, equipment)	Prevention of faecal contamination of milk and introduction of salmonella, STEC, <i>L. monocytogenes</i> and <i>S. aureus</i>	FACE (2016), SCA (2015) Espié et al. (2006)
Effective cleaning and sanitation in dairy facilities	Inactivation of STEC, <i>Listeria</i> biofilms	Sharma et al. (2005)
Microbiological control of feed: pasture/dry hay	Prevents exposure of lactating animals to <i>Listeria monocytogenes</i> in silage, which can lead to shedding of <i>L. monocytogenes</i> in milk. Use of distillers grains in feed increases STEC shedding in milk	Lekkas (2016) Dewell (2005)
Identification of farm niches where pathogens can survive	Leads to overall greater farm hygiene and reduction of incidence of <i>L. monocytogenes</i> and <i>S. aureus</i>	D'Amico and Donnelly (2010) Arimi et al. (1997)
Disposable in-line filters (filter socks or disposable in-line filters)	Trap somatic cells and debris that can be sources of pathogens; microbiological testing of milk filters for pathogens (<i>L. mono</i> , salmonella, STEC) provides better assurance of milk safety compared to milk testing. Filter testing improves detection of STEC	D'Amico and Donnelly (2010) Lekkas (2016) FSAI 2015 Lambertini et al. (2015)
Monitoring Milk Temperature: Use of time/temperature controls during milk handling, storage and transportation Milk should be cooled to <4°C immediately after milking. Milk must be cooled immediately to no more than 8°C in the case of daily collection, or not more than 6°C if collection is not daily. Ensure that milk doesn't exceed 10°C during transport FBOs must ensure that, upon acceptance at processing establishment, milk is quickly cooled to not more than 6°C and kept at that temperature until processed Storage for <24 h at 3°C	Assures milk hygiene and safety Inadequate milk storage temperature implicated in outbreaks	SCA (2015) FSANZ (2005 ⁵⁵) & (2009 ⁵⁶) FSAI (2015) Regulation EC 853/2004 Reid (2001)
Rapid transformation⁵⁷ of milk	Transformation within 4 hours limits potential for growth of pathogens including salmonella, <i>L. mono</i> , STEC, and <i>S. aureus</i>	SCA (2015)
Testing to verify raw milk quality (NB also refer to Table 8).	TARGET: Plate count at 30°C <10,000/ml cow and other species, <i>E. coli</i> <100 cfu/ml. Periodic, risk based testing (e.g. monthly ⁵⁸) for salmonella, <i>L. mono</i> & STEC O157 recommended.	SCA (2015) FSANZ (2009 ⁵⁹) FSANZ (2015) ⁶⁰

⁵⁵ Draft Assessment Report Application A499 to permit the sale of Roquefort Cheese. 23 March 2005. FSANZ

⁵⁶<https://www.foodstandards.gov.au/code/proposals/documents/p1007%20ppps%20for%20raw%20milk%201ar%20s%20d1%20cow%20milk%20risk%20assessment.pdf>

⁵⁷ Transformation is the process of turning milk into cheese.

⁵⁸ Monthly is a typical interval. If a cheesemaker tests for pathogens every six months and finds positive results, cheese made during the previous six months may be contaminated and may need to be discarded. With monthly testing, only a month's worth of production would be affected. Rather than being prescriptive, each cheesemaker needs to design a food safety plan that works for them within their constraints of cost and the level of safety they wish to assure.

⁵⁹<https://www.foodstandards.gov.au/code/proposals/documents/p1007%20ppps%20for%20raw%20milk%201ar%20s%20d1%20cow%20milk%20risk%20assessment.pdf>

2.3.4. Control of the Processing Environment

In addition to raw milk management, the microbiological safety of the cheese processing environment must be controlled through sanitation and GHP. *L. monocytogenes* is the environmental pathogen of most significance in the cheese manufacturing environment, and control of *L. monocytogenes* through effective sanitation and hygiene will likely help in the control of other pathogens and spoilage flora.

The incidence and ecology of *Listeria* spp. in farmstead cheese processing environments was assessed through environmental sampling conducted in nine facilities in a study over a 6-week period (D'Amico and Donnelly 2009). Environmental samples (450) were collected with environmental sponges from both food contact (FCS) and non-food contact (NFCS) surfaces, and examined for the presence of *Listeria* spp. using four detection/isolation protocols (with different isolates recovered depending on the method used). Thirty three sites tested positive for *Listeria* spp. including five FCS and twenty-eight non-FCS. *L. monocytogenes* accounted for 20% (142/710) of the isolates from 15 of the 53 (28.3%) *Listeria* spp. positive sites.

L. monocytogenes isolates were characterised by automated *EcoRI* subtyping to examine strain diversity within and between plants over time as well as the impact of enrichment media utilised. While most subtypes were consistently isolated by all enrichment procedures, DUP-10144 (of a unique subtype) was solely isolated with protocols that utilise *Listeria* Repair Broth (LRB) in primary enrichment showing that certain subtypes will escape detection unless enrichment conditions are modified to allow their repair and recovery, although most subtypes are routinely recovered using standard enrichment procedures that do not use LRB. Eighty-eight isolates, recovered from a single facility, were differentiated into four subtypes (19171, 10144, 19157, and 1042B), in 3 ribogroups. Sixty-nine (78.4%) of these were identified as DUP-1042B, a known lineage I “epidemic subtype” which has caused notable outbreaks due to the consumption of pasteurised milk and Mexican style soft cheese among others (Neves et al. 2008). DUP-1042B was the predominant isolate from 8/9 positive sites including two FCS. The presence of this identical subtype on both FCS and non-FCS suggests cross-contamination within the plant.

These findings emphasise the important role for cheesemakers of environmental contaminants as sources of finished product contamination. While the persistence of specific subtypes in processing facilities has been shown, shifts in population subtypes between samplings in this study demonstrates recontamination of a single site with new subtypes. Furthermore, subtypes of isolates recovered in 2004 differ from those isolated in 2008 from the same plant. Raw milk was not the likely contamination source as raw milk isolate subtypes from the same farm did not match those from within the processing environments (likely sources were soil and manure tracked in via footwear). Analysis of the distribution of subtypes between plants revealed that each facility had

⁶⁰ <https://www.foodstandards.gov.au/code/proposals/Documents/P1022-Raw-milk-prods-AppR-SD1.pdf>

unique contamination subtypes. The use of molecular subtyping can provide useful information on the ecology of different *L. monocytogenes* strains within and between food processing environments, and information can be used to develop improved control strategies. It is critical for artisan cheese producers to conduct routine environmental surveillance to ensure that ageing facilities used for artisan cheese production do not harbor *L. monocytogenes*.

Dalmasso and Jordan (2013) found that NFCS and the outside environment posed a risk to farmhouse cheese of contamination with *L. monocytogenes*. The authors provided advice to farmhouse cheesemakers concerning control strategies to prevent dissemination of *L. monocytogenes* to the processing environment that included use of adequate cleaning and disinfection and altered workflows to control foot traffic (and therefore dissemination of *Listeria*). Upon application of these recommendations, a decrease in *L. monocytogenes* incidence occurred. The study showed the value of effective environmental sampling plans coupled with appropriate and timely corrective actions to improve food safety in the cheesemaking environment.

The EU (FACEnetwork 2016) has developed the “[European Guide for Good Hygiene Practices in the production of artisanal cheese and dairy products](#)”, which targets farmhouse and artisan producers. The condition of the premises was identified in previous research as a factor affecting presence of *L. monocytogenes*. Preventive measures recommended by FACEnetwork include control of the quality of animal feed (at the farm level) and water, cleaning of equipment and establishment of general hygiene practices on farms and in processing areas.

2.3.5. Controlling factors to be utilised during the cheesemaking process across different cheese types commonly produced across Scotland, by category, and evidence to support those controls

For each Codex category of soft, semi-soft, semi-hard and hard cheese, a flow chart has been constructed to show basic steps in cheesemaking for cheeses in these categories, along with microbiological requirements and key controlling factors for the pathogens of concern in these cheese types, along with evidence to support these controls (Figures 5-9). These are intended to provide examples and references only. All HACCP plans should be designed individually by businesses specifically for their own product(s) and processes.

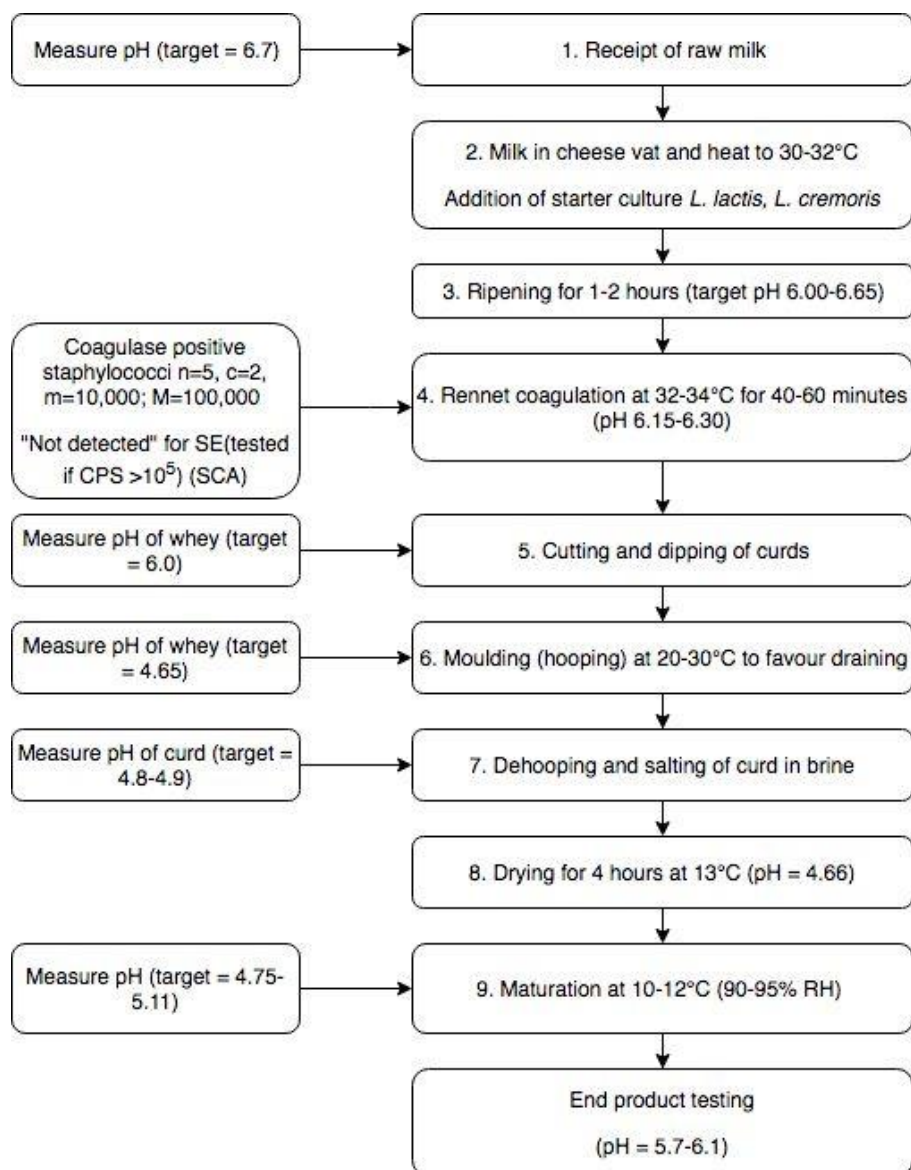
As emphasised by the SCA, certain cheeses present a greater risk to microbiological safety therefore, in developing HACCP plans, the same CCPs cannot be applied to all varieties of cheese (SCA 2015). During cheesemaking, the main shifts in the microbial composition of cheese occur during curd production and ripening, indicating that the key driving forces for microbial growth in cheese are pH and salt content (Irlinger et al. 2015; Fuka et al. 2013).

Cheesemaking involves a combination of hurdles that influence the growth and survival of pathogenic microorganisms. It is often this combination of hurdles, rather than an individual processing step or physicochemical property, that has the greatest impact on pathogen survival in raw milk cheese⁶¹. The SCA Assured Code of Practice (ACoP) discusses the legal requirement for HACCP in the UK and the need to control hazards through identification of Critical Control Points (CCPs). CCPs must be monitored each time cheese is made to ensure that the cheesemaking process is controlling identified hazards, or whether corrective actions are needed to bring the cheesemaking process back under control.

⁶¹<https://www.foodstandards.gov.au/code/proposals/documents/P1007%20PPPS%20for%20raw%20milk%201AR%20SD3%20Cheese%20Risk%20Assessment.pdf>

Figure 5: Example process flowchart for soft cheese made from unpasteurised milk^{62,63}

Example: Soft bloomy rind cheese e.g. Camembert (see Table 2, for physicochemical characteristics of each cheese type)⁶⁴



⁶² Please note: these are typical values for each cheese type and are for information only. The cheesemaking process is highly variable and these flowcharts are not meant to be prescriptive.

⁶³ Please note: there are no soft cheeses currently being produced in Scotland from unpasteurised milk, and it is unlikely that it would be possible to produce them safely due to their physicochemical properties being inherently dangerous.

⁶⁴ Guidance in these tables is specific for the type of cheese described and not generally applied to all cheeses.

Table 10: Typical physicochemical characteristics of mature Camembert

Mature Camembert	Mean	Range
pH	6.0	5.7 – 6.1
Water activity (A_w)	0.97	0.96 – 0.98
Salt content	1.8%	1.5 - 2.8%

Table 11: Microbiological Criteria from Regulation (EC) 2073/2005

Food category	Micro-organisms/ their toxins, metabolites	Sampling Plan ⁶⁵		Limits ⁶⁶		Analytical reference method ⁶⁷	Stage where the criterion applies
		n	c	m	M		
1.2 Ready-to-eat foods able to support the growth of <i>L. monocytogenes</i> , other than those intended for infants and for special medical purposes	<i>Listeria monocytogenes</i>	5	0	100 cfu/g ⁶⁸		EN/ISO 11290-2 ⁶⁹	Products placed on the market during their shelf-life
		5	0	Absence in 25g ⁷⁰		EN/ISO 11290-1	Before the food has left immediate control of the food business operator, who has produced it
1.11 Cheeses, butter and cream made from raw milk or milk that has undergone a lower heat treatment than pasteurisation ⁷¹	Salmonella	5	0	Absence in 25g		EN/ISO 6579	Products placed on the market during their shelf-life.
1.21 Cheeses, milk powder and whey powder, as referred to in the coagulase-positive staphylococci criteria in Chapter 2.2	Staphylococcal enterotoxins	5	0	Not detected in 25g		European screening method of the CRL for coagulase positive staphylococci ⁷²	Products placed on the market during their shelf-life.

⁶⁵ N = number of units comprising the sample; c = number of sample units giving values over m or between m and M.

⁶⁶ For 1.2 m=M

⁶⁷ The most recent addition of the standard shall be used.

⁶⁸ This criterion applies if the manufacturer is able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 cfu/g throughout the shelf life. The operator may fix intermediate limits during the process that should be low enough to guarantee that the limit of 100 cfu/g is not exceeded at the end of shelf-life.

⁶⁹ 1ml of inoculum is plated on a Petri dish of 140 mm diameter or on three Petri dishes of 90 mm diameter.

⁷⁰ This criterion applies to products before they have left the immediate control of the producing food business operator, when he is not able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 cfu/g throughout the shelf-life.

⁷¹ Excluding products where the manufacturer can demonstrate to the satisfaction of the competent authorities that, due to the ripening time and a_w of the product where appropriate, there is no salmonella risk.

⁷² Reference: (Hennekinne et al. 2003) Hennekinne et al., J. AOAC Internat. Vol. 86, No 2, 2003.

Table 11 (continued): Microbiological Criteria from Regulation (EC) 2073/2005

Food category	Micro-organisms	Sampling Plan ₁		Limits		Analytical reference method ₃	Stage where the criterion applies	Action in case of unsatisfactory results
		n	c	m	M			
2.2.3 Cheese made from raw milk	Coagulase-positive staphylococci	5	2	10 ⁴ cfu/g	10 ⁵ cfu/g	EN/ISO 6888-2	At the time during the manufacturing process when the number of staphylococci is expected to be highest	Improvements in production hygiene and selection of raw materials. If values >10 ⁵ cfu/g are detected, the cheese batch has to be tested for staphylococcal enterotoxins.

Table 12: Microbiological Criteria for pathogens in soft cheese made from unpasteurised milk

Pathogen	Limit	Source	Stage where criteria applies
<i>E. coli</i> O157/ STEC	Absence in 25g	HPA Guidelines Draft UK policy position on STEC SCA ACOP 2015	End of production (EOP)
<i>Staphylococcus aureus</i>	n=5; c=2; m=10,000; M=100,000	Regulation EC 2073/2005	Sample curd at the point where levels are likely to be highest

Table 13: Microbiological Criteria for indicators in soft cheese made from unpasteurised milk

Indicator	Limit	Source	Stage where criteria applies
Enterobacteriaceae ⁷³	<10,000 cfu/g	SCA ACOP 2015	EOP
	Unsatisfactory if > 10,000 cfu/g Borderline if 100 -10,000 cfu/g	UK HPA Guidelines	EOP
<i>E. coli</i>	< 10,000 cfu/g	SCA ACOP 2015	EOP

⁷³ The criterion does not apply to cheeses ripened using a culture of *Hafnia alvei* or *Proteus vulgaris*.

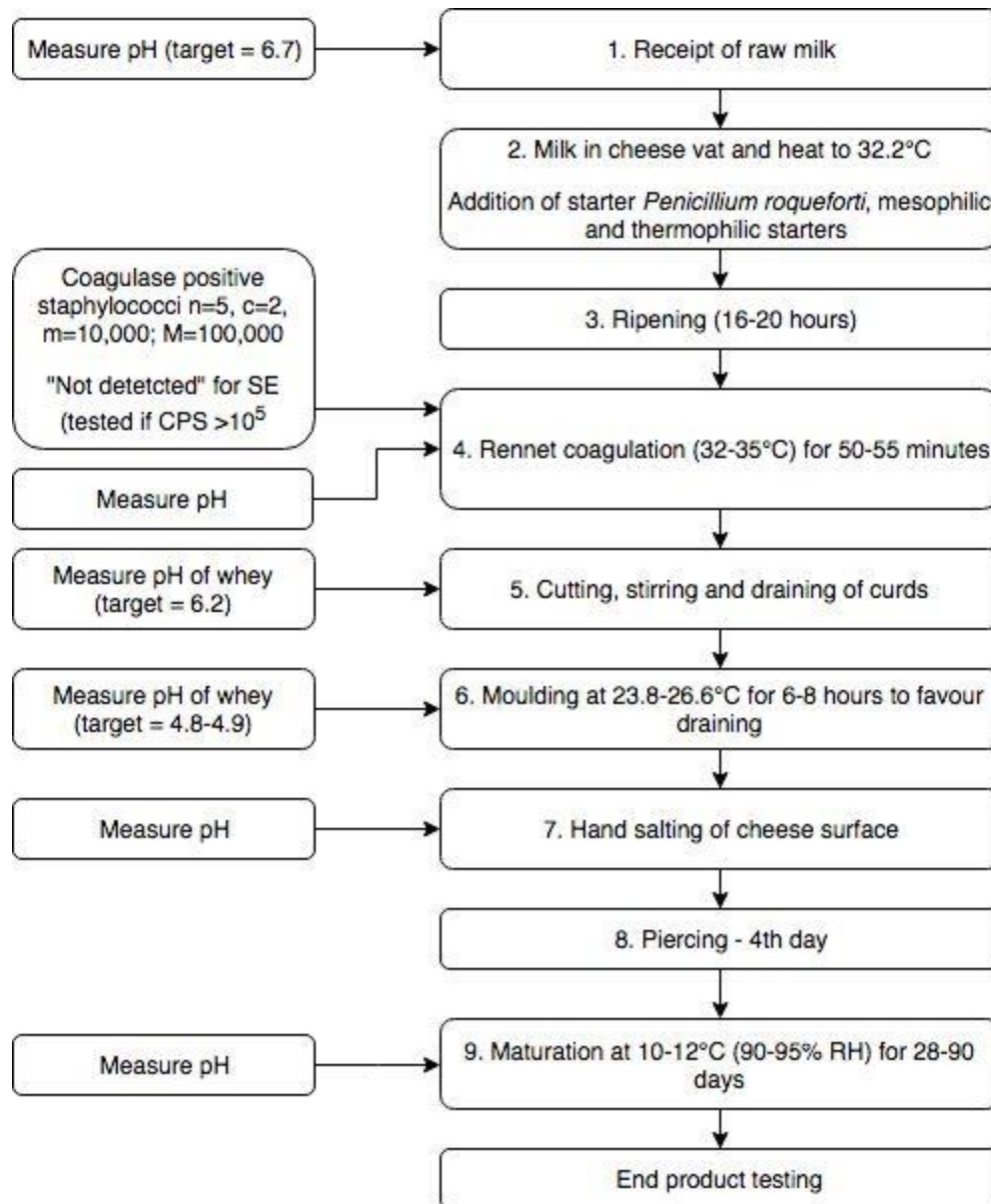
Table 14: Key Factors for Managing Microbiological Risk during Production of Soft Cheese. Example: bloomy rind cheese (Camembert) made from unpasteurised milk

Controlling parameter	What it facilitates	Supporting evidence
Initial Milk Temperature 8°C, not to exceed 10°C; storage for <24 h at 3°C Microbiological status of incoming raw milk: <i>S. aureus</i> <100 cfu/ml; Coliforms <100cfu/ml, <i>E. coli</i> <100 cfu /ml, Bactoscan count <12(000)/ml.	Assures milk hygiene and safety Controls growth of <i>L. monocytogenes</i> , salmonella, <i>E. coli</i> and coagulase positive staphylococci.	SCA ACoP (2015), Regulation EC 853/2004
Establishment of a target acidity schedule consisting of 4 key measurements: (i) milk initially in vat (pH 6.7); (ii) after starter addition to milk; (iii) whey pH at draining (target 6.0); (iv) curd pH at molding (target 4.65).	Assures acid development by starter and aids with control of cheese composition. Controls undesirable fluctuations in quality that compromises safety. Rapid acidification from pH 6.5 to 5.0 within 6-8 hours then pH 4.8 within 24hrs can achieve inactivation of salmonella if pH reaches 4.8	Kindstedt (2005) FSANZ (2005) ⁷⁴ Montet (2009)
Process hygiene controls Testing for coagulase positive staphylococci during manufacture when counts are expected to be the highest. n=5; c=2; m=10,000; M=100,000.	Assures production hygiene and microbiological quality of raw materials; absence of enterotoxins; activity of starter culture	Regulation EC 2073/2005
Salting and measurement of salt in moisture; final content 1.8-2.0%	Assures correct moisture levels Minor hurdle for <i>L. monocytogenes</i> and <i>S. aureus</i>	Kindstedt (2005)
Maturation 10-12°C, 90-98% relative humidity Targets: Mean pH at beginning of maturation 4.75-5.11; mean pH 6.0 at end of maturation (range 5.7-6.1) Aw 0.97 (range 0.96-0.98), salt content 1.8% (range 1.5-2.8%).	pH reversion during maturation as a result of <i>Penicillium</i> growth allows growth of <i>L. monocytogenes</i> ; growth parallels pH increase. Post process environmental contamination must be controlled and verified through environmental testing	Ryser and Marth (1987) D'Amico et al. (2008) D'Amico and Donnelly (2009)
Food Safety Criteria Testing for absence of salmonella/25g cheese at end of production (EOP) & during shelf life	Food safety assurance	Regulation EC 2073/2005
Absence of <i>L. monocytogenes</i> /25g cheese at EOP before the food has left the immediate control of the FBO who produced it or <100 cfu/g during the shelf life	Food safety assurance; verifies that <i>L. monocytogenes</i> in raw milk has been controlled by the cheesemaking process and no post-process recontamination has occurred. Testing every batch of cheese for <i>L. monocytogenes</i> achieves a mean level of safety higher than use of pasteurised milk alone.	Regulation EC 2073/2005 FDA/Health Canada 2015
Absence of <i>E. coli</i> O157:H7/25g at EOP and during the shelf life	Food safety verification	SCA ACoP (2015)/HPA guidelines

⁷⁴ Draft Assessment Report Application A499 to permit the sale of Roquefort Cheese. 23 March 2005. Food Standards Australia New Zealand
<https://view.officeapps.live.com/op/view.aspx?src=https%3A%2F%2Fwww.foodstandards.gov.au%2Fcode%2Fapplications%2Fdocuments%2FA499%2520Roquefort%2520DAR.doc>

Figure 6: Example Process flowchart for semi-soft cheese made from unpasteurised milk⁷⁵

Example: Fourme D'Ambert



⁷⁵ Please note: these are typical values for each cheese type and are for information only. The cheesemaking process is highly variable and these flowcharts are not meant to be prescriptive.

Table 15: Typical physicochemical characteristics of mature Fourme D'Ambert (Controlling factors at dispatch)

Fourme D'Ambert	Mean	Range
pH	5.8	5.5 – 6.5
Final pH after 8 weeks	-	6.0 - 6.25
Water activity (A_w)	0.95	0.94 – 0.96
Salt content	2.5%	-
Salt in moisture	5.6%	5.5 – 5.7%

For Microbiological Criteria from Regulation (EC) 2073/2005, please see Table 11 above.

Table 16: Microbiological Criteria for pathogens in semi-soft cheese made from unpasteurised milk

Pathogen	Limit	Source	Stage where criteria applies
<i>E. coli</i> O157/ STEC	Absence in 25g	HPA Guidelines Draft UK policy position on STEC	End of production (EOP)
<i>Staphylococcus aureus</i>	n=5; c=2; m=10,000; M=100,000	EC 2073/2005	Sample curd at the point where levels are likely to be highest

Table 17: Microbiological Criteria for hygiene indicators in semi-soft cheese made from unpasteurised milk

Indicator	Limit	Source	Stage where criteria applies
Enterobacteriaceae	Mould-ripened soft and semi soft cheese < 10,000 cfu/g Washed-rind soft and semi-soft cheeses <100,000 cfu/g	SCA ACOP 2015	EOP
	Unsatisfactory if > 10,000 cfu/g Borderline if 100 -10,000 cfu/g	UK HPA Guidelines	EOP
<i>E. coli</i>	< 10,000 cfu/g	SCA ACOP 2015	EOP

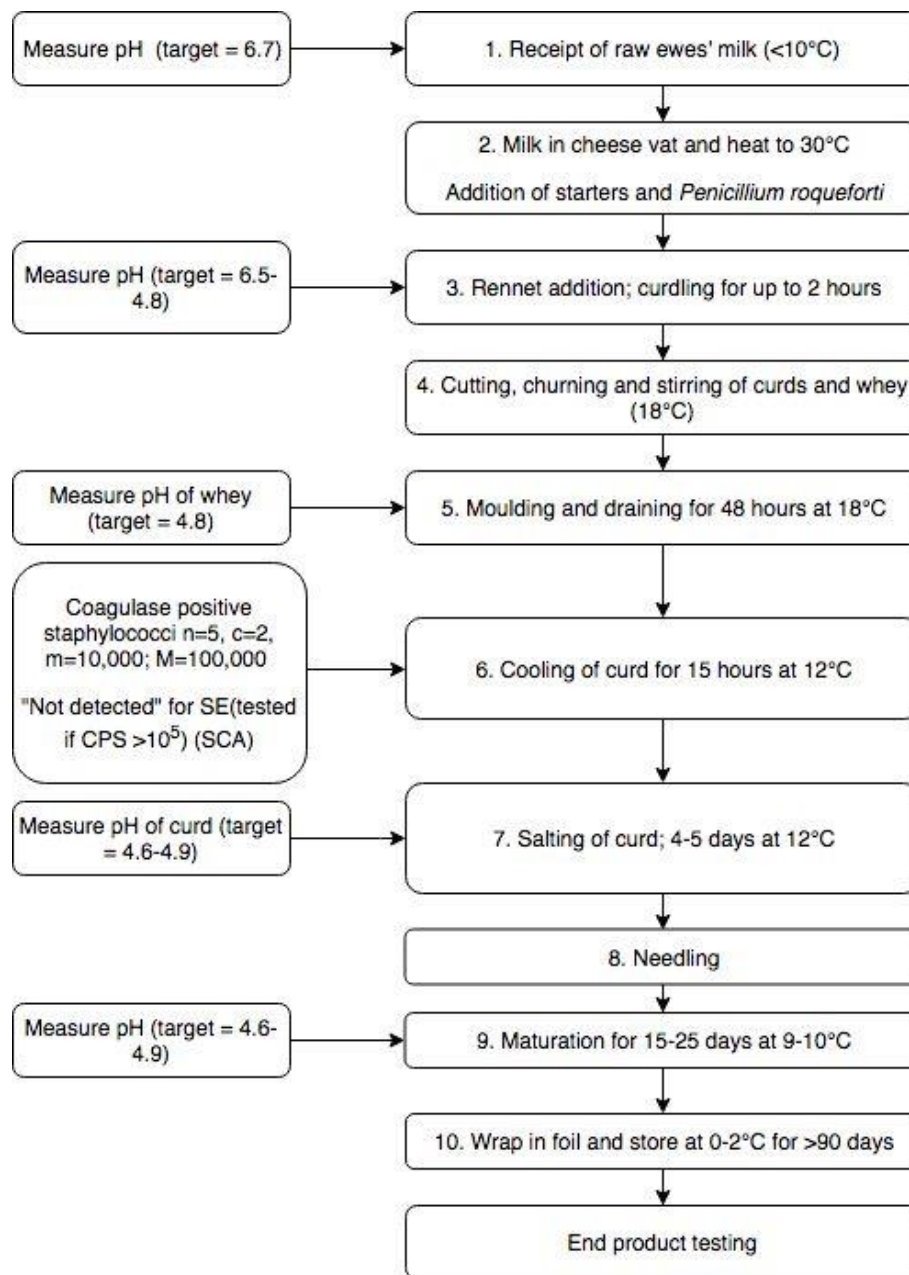
Table 18: Key Factors for Managing Microbiological Risk during Production of Semi Soft Cheese. Example Fourme D'Ambert made from unpasteurised milk

Controlling parameter	What it facilitates	Supporting evidence
Feed Produced from cows fed on grass; restriction of proportion of maize silage in winter (50%)	Microbiological safety of raw milk;	Bord et al. (2015)
Initial Milk Temperature not to exceed 10°C; storage for <24 h at 3°C Microbiological status of incoming raw milk: <i>S. aureus</i> <100 cfu/ml; Coliforms <100cfu/ml, <i>E. coli</i> <100 cfu /ml, Bactoscan count <12(000)/ml.	Assures milk hygiene and safety	SCA ACOP 2015 Regulation EC 853/2004
Establishment of a target acidity schedule consisting of 5 key measurements (milk initially in vat; after starter addition to milk; whey pH at draining; curd pH at milling; curd pH at molding and pressing).	Assures acid development by starter and aids with control of cheese composition. Controls undesirable fluctuations in quality that compromises safety Rapid acidification from pH 6.5 to 5.3 within 6-8 hrs; pH 4.8 within 24h. Inactivation of salmonella when pH reaches 4.8	Kindstedt (2005) FSANZ (2005) ⁷⁶
Process hygiene controls Testing for coagulase positive staphylococci during manufacture when counts are expected to be the highest. n=5; c=2; m=10,000; M=100,000.	Assures production hygiene and microbiological quality of raw materials; absence of enterotoxins; activity of starter culture	Regulation EC 2073/2005
Salting and measurement of salt in moisture; final salt content 2.5%; 5.6% S/M	Assures correct moisture levels to control microbial growth.	Kindstedt (2005)
Maturation 10-12°C, 90-95% RH for 28- 90 days; Targets: Mean pH of 5.8 (range 5.5-6.5) at end of production; Aw 0.95 (0.94-0.96), salt content 2.5%.	Facilitates reduction of salmonella, <i>E. coli</i> O157:H7 and <i>Listeria</i> during ageing.	Papageorgiou and Marth (1989)
Food safety criteria Absence of salmonella/25g cheese at end of production (EOP) and during the shelf life	Food safety assurance	EU Regulation 2073/2005
Absence of <i>L. monocytogenes</i> / 25g cheese at EOP before the food has left the immediate control of the FBO who produced it or <100 cfu/g during shelf life	Food safety assurance/hard cheese	EU Regulation 2073/2005
Absence of <i>E. coli</i> O157:H7/25g at EOP and during the shelf life	Food safety assurance	SCA ACOP 2015 HPA RTE guidelines

⁷⁶ Draft Assessment Report Application A499to permit the sale of Roquefort Cheese. 23 March 2005. Food Standards Australia New Zealand

Figure 7: Example Process flowchart for semi-hard cheese made from unpasteurised milk⁷⁷

Example: Roquefort



⁷⁷ Please note: these are typical values for each cheese type and are for information only. The cheesemaking process is highly variable and these flowcharts are not meant to be prescriptive.

Table 19: Typical physicochemical characteristics of mature Roquefort (FSANZ (2005)⁷⁸; FSANZ (2009)⁷⁹)

Roquefort	Mean	Range
pH	5.5	5.5-6.5
Water activity (A_w)	0.92	0.92 – 0.94
Salt content	3.0%	2.0-4.3

For Microbiological Criteria from Regulation (EC) 2073/2005, please see Table 11 above.

Table 20: Microbiological Criteria for pathogens in semi-hard cheese made from unpasteurised milk

Pathogen	Limit	Source	Stage where criteria applies
<i>E. coli</i> O157/ STEC	Absence in 25g	HPA Guidelines Draft UK policy position on STEC	EOP
<i>Staphylococcus aureus</i> (CPS)	n=5; c=2; m=10,000; M=100,000	Regulation EC 2073/2005	When CPS levels are expected to be the highest

Table 21: Microbiological Criteria for hygiene indicators in semi-hard cheese made from unpasteurised milk

Indicator	Limit	Source	Stage where criteria applies
Enterobacteriaceae	< 100 cfu/g	SCA ACOP 2015	EOP
	Unsatisfactory if > 10,000 cfu/g Borderline if 100 -10,000 cfu/g	UK HPA Guidelines	EOP
<i>E. coli</i>	< 100 cfu/g	SCA ACOP 2015	EOP

⁷⁸ http://www.foodstandards.gov.au/code/applications/Documents/A499_Roquefort_FAR_FINALv2.doc

⁷⁹

<https://www.foodstandards.gov.au/code/proposals/documents/P1007%20PPPS%20for%20raw%20milk%201AR%20SD3%20Cheese%20Risk%20Assessment.pdf>

Table 22: Key Factors for Managing Microbiological Risk during Production of Semi-Hard Cheese Example: Roquefort made from unpasteurised milk

Controlling parameter	What it facilitates	Supporting evidence
Initial Milk Temperature 8°C, not to exceed 10°C; storage for <24 h at 3°C SCA: Microbiological status of incoming raw milk: <i>S. aureus</i> <100 cfu/ml; Coliforms <100cfu/ml, <i>E. coli</i> <100 cfu /ml, Bactoscan count <12(000)/ml. FSANZ: <500 <i>E. coli</i> /ml ⁸⁰ , absence of <i>L. monocytogenes</i> and salmonella	Assures milk hygiene and safety	SCA, Regulation EC 853/2004 FSANZ 2005 ⁸¹ (pg. 135, Appendix A499)
Establishment of a target acidity schedule consisting of 5 key measurements (milk initially in vat; after starter addition to milk; whey pH at draining; curd pH at milling; curd pH at molding and pressing).	Assures acid development by starter and aids with control of cheese composition. Controls undesirable fluctuations in quality that compromises safety Rapid acidification from pH 6.5 to 5.0 within 6-8 hrs; pH 4.8 within 24h. Inactivation of salmonella when pH reaches 4.8	Kindstedt 2005 FSANZ 2005 ⁸²
Process hygiene controls Testing for coagulase positive staphylococci during manufacture when counts are expected to be the highest. n=5; c=2; m=10,000; M=100,000.	Assures production hygiene and microbiological quality of raw materials; absence of enterotoxins; activity of starter culture	Regulation EC 2073/2005
Salting and measurement of salt in moisture; final salt content 3%	Assures correct moisture levels to control microbial growth.	Kindstedt (2005)
Maturation 9-10°C, 85-90% humidity for a minimum of 90 days; Targets: Mean pH of 5.5-6.0 (range 5.5-6.5) at end of production; Aw 0.92, salt content 3%.	Facilitates reduction of salmonella, <i>E. coli</i> O157:H7 and <i>Listeria</i> during ageing. STEC declines during maturation due to desiccation of curd; populations of <i>E. coli</i> O157:H7 reaching levels of >3,000 cfu/g declined following salting and were not detected through enrichment beyond 90 days of maturation	Papageorgiou and Marth (1989) FSANZ 2005 ⁷⁰
Food safety criteria Absence of salmonella/25g cheese at end of production (EOP) and during shelf life	Food safety assurance	Regulation EC 2073/2005
Absence of <i>L. monocytogenes</i> / 25g cheese at EOP before the food has left the immediate control of the FBO who produced it	Food safety assurance/hard cheese	Regulation EC 2073/2005
Absence of <i>E. coli</i> O157:H7/25g at EOP and during shelf life	Food safety assurance	SCA ACOP 2015

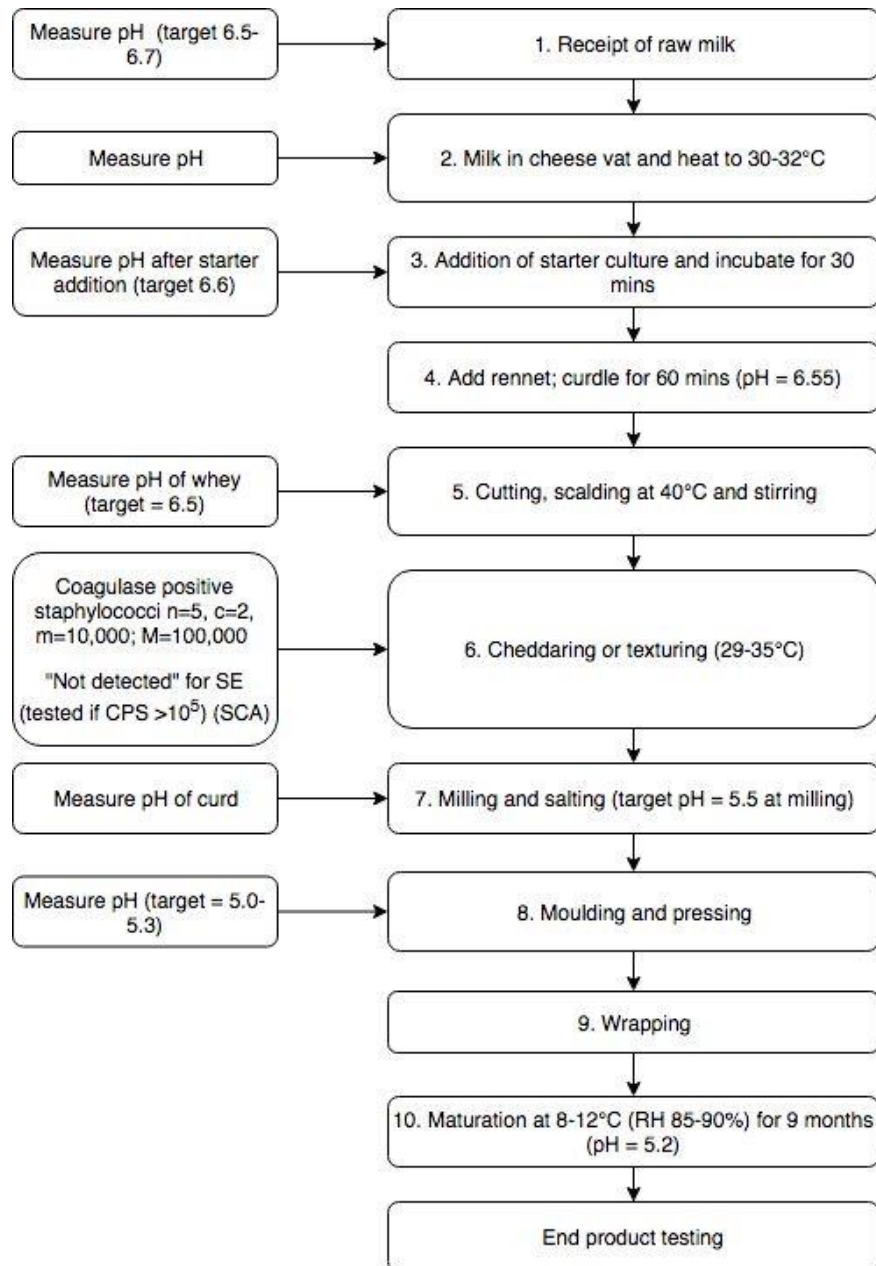
⁸⁰ This is a specific recommendation for Roquefort as per the FSANZ risk assessment

⁸¹ http://www.foodstandards.gov.au/code/applications/Documents/A499_Roquefort_FAR_FINALv2.doc

⁸² Draft Assessment Report Application A499to permit the sale of Roquefort Cheese. 23 March 2005. FSANZ

Figure 8: Example process flowchart for hard cheese made from unpasteurised milk⁸³

Example: Cheddar



⁸³ Please note: these are typical values for each cheese type and are for information only. The cheesemaking process is highly variable and these flowcharts are not meant to be prescriptive.

Table 23: Typical physicochemical characteristics of mature Cheddar cheese

Mature Cheddar Cheese	Mean	Range
pH	5.2	4.9 – 5.3
Water activity (A_w)	0.93	0.92 – 0.959
Salt content	2.0%	1.5 – 2.5%
Salt in moisture	-	4.5 – 5.55%

For Microbiological Criteria from Regulation (EC) 2073/2005, please see table 11 above.

Table 24: Microbiological Criteria for pathogens in hard cheese made from unpasteurised milk

Pathogen	Limit	Source	Stage where criteria applies
<i>E. coli</i> O157/ STEC	Absence in 25g	HPA Guidelines Draft UK policy position on STEC	EOP
<i>Staphylococcus aureus</i> /CPS	n=5; c=2; m=10,000; M=100,000.	EC 2073/2005	When CPS levels are expected to be the highest

Table 25: Microbiological Criteria indicators for hard cheese made from unpasteurised milk

Indicator	Limit	Source	Stage where criteria applies
Enterobacteriaceae	< 100 cfu/g	SCA ACOP 2015	EOP
	Unsatisfactory if > 10,000 cfu/g Borderline if 100 -10,000 cfu/g	UK HPA Guidelines	EOP
<i>E. coli</i>	< 100 cfu/g (hard cheese)	SCA ACOP 2015	EOP

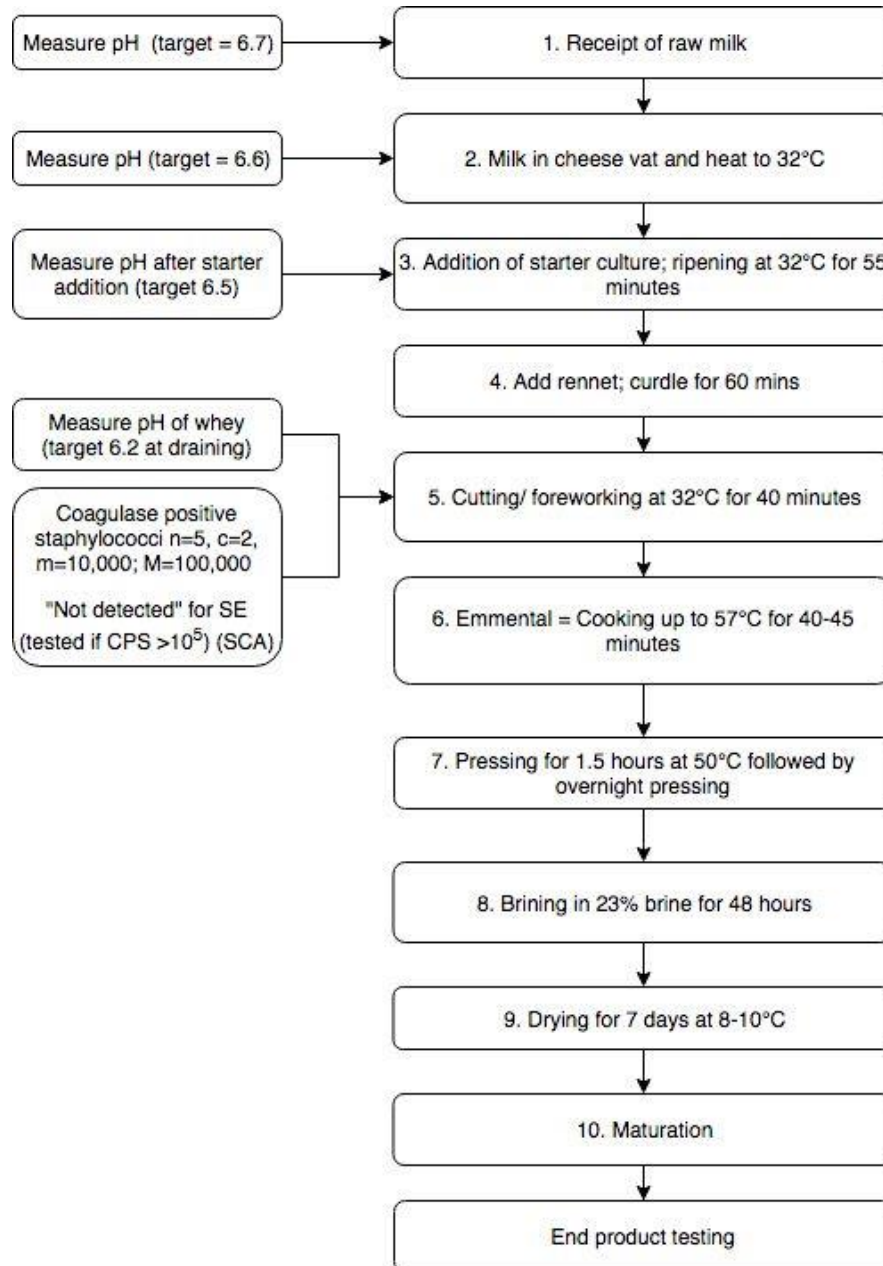
Table 26: Key Factors for Managing Microbiological Risk during Production of Hard Cheese

Example: Cheddar made from unpasteurised milk

Controlling parameter	What it facilitates	Supporting evidence
Initial Milk Temperature 6°C, not to exceed 10°C Microbiological status of incoming raw milk: <i>S. aureus</i> <100 cfu/ml; Coliforms <100cfu/ml, <i>E. coli</i> <100 cfu /ml, Bactoscan count <12(000)/ml.	Assures milk hygiene and safety Prevents/slows growth of <i>S. aureus</i> , <i>L. monocytogenes</i> , salmonella, <i>E. coli</i>	SCA ACOP 2015, Regulation EC 853/2004
Establishment of a target acidity schedule consisting of 5 key measurements (milk initially in vat; after starter addition to milk; whey pH at draining; curd pH at milling; curd pH at molding and pressing).	Assures acid development by starter and aids with control of cheese composition. Controls undesirable fluctuations in quality that compromises safety	Kindstedt (2005)
Process hygiene controls Testing for coagulase positive staphylococci during manufacture when counts are expected to be the highest. n=5; c=2; m=10,000; M=100,000. NB <i>Sa</i> <1000/g (SCA)	Assures production hygiene and microbiological quality of raw materials; absence of enterotoxins; activity of starter culture	Regulation EC 2073/2005 SCA ACOP 2015
Salting and measurement of salt in moisture	Assures correct moisture levels to control microbial growth; minor hurdle for <i>L. mono</i> and <i>S. aureus</i>	Kindstedt (2005)
Maturation 8-12°C, 85-90% humidity for a minimum of 60 days; average 9 months. Targets: Mean pH of 5.2 (range 4.9-5.3) at end of production; Aw 0.93, salt content 2%.	Facilitates reduction of salmonella, <i>E. coli</i> O157:H7 and <i>Listeria</i> during ageing.	Ryser and Marth (1987) Goepfert et al. (1968) D'Amico et al. (2010) Reitsma and Henning (1996)
Process Safety Controls: Absence of <i>Salmonella</i> /25g cheese at end of production (EOP) Absence of <i>L. monocytogenes</i> / 25g cheese at EOP or <100 cfu/g at end of shelf life Absence of <i>E. coli</i> O157:H7/25g at EOP and during shelf life Enterobacteriaceae <100 cfu/g <i>E. coli</i> <100 cfu/g	Food safety assurance Food safety assurance/hard cheese Food safety assurance Process hygiene Process hygiene and safety	Regulation EC 2073/2005 Regulation EC 2073/2005 Regulation EC 2073/2005 SCA ACOP 2015 SCA Hard Cheese

Figure 9: Example Process flowchart for hard cheese made from unpasteurised milk⁸⁴

Example: Cooked curd cheese, Emmental



⁸⁴ Please note: these are typical values for each cheese type and are for information only. The cheesemaking process is highly variable and these flowcharts are not meant to be prescriptive.

Table 27: Typical physicochemical characteristics of mature hard cheese

Mature Hard Cheese (e.g. Emmental)	Mean	Range
pH	5.6	5.4-5.8
Water activity (A_w)	0.97	0.92–0.97
Salt content	0.7%	0.7-1.1%

For Microbiological Criteria from Regulation (EC) 2073/2005, please see table 11 above.

Table 28: Microbiological Criteria for pathogens in hard cheese made from unpasteurised milk

Pathogen	Limit	Source	Stage where criteria applies
<i>E. coli</i> O157/ STEC	Absence in 25g	HPA Guidelines Draft UK policy position on STEC	EOP
<i>Staphylococcus aureus</i> (CPS)	n=5; c=2; m=10,000; M=100,000	Regulation EC 2073/2005	When CPS levels are expected to be the highest

Table 29: Microbiological Criteria indicators in hard cheese made from unpasteurised milk

Indicator	Limit	Source	Stage where criteria applies
Enterobacteriaceae	< 100 cfu/g	SCA ACOP 2015	EOP
	Unsatisfactory if > 10,000 cfu/g Borderline if 100 -10,000 cfu/g	UK HPA Guidelines	EOP
<i>E. coli</i>	< 100 cfu/g (hard cheese)	SCA ACOP 2015	End of maturation

Table 30: Key Factors for Managing Microbiological Risk during Production of Hard Cheese. Example: Emmental made from unpasteurised milk

Controlling parameter	What it facilitates	Supporting evidence
Feeding of natural forage: grass in summer, hay in winter, no silage*	Microbiological quality and safety; controls late blowing defect from bacterial spores; also controls <i>L. monocytogenes</i>	Bachman and Spahr (1995)
Initial Milk Temperature 6°C, not to exceed 10°C. Microbiological status of incoming raw milk: <i>S. aureus</i> <100 cfu/ml; Coliforms <100cfu/ml, <i>E. coli</i> <100 cfu /ml, Bactoscan count <12(000)/ml.	Assures milk hygiene and safety Prevents/slows growth of <i>S. aureus</i> , <i>L. monocytogenes</i> , salmonella, <i>E. coli</i>	SCA (2015) Regulation EC 853/2004
Establishment of a target acidity schedule consisting of 5 key measurements (milk initially in vat; after starter addition to milk; whey pH at draining; curd pH at milling; curd pH at molding and pressing).	Assures acid development by starter and aids with control of cheese composition. Controls undesirable fluctuations in quality that compromises safety	Kindstedt (2005)
Process hygiene controls Testing for coagulase positive staphylococci during manufacture when counts are expected to be the highest. n=5; c=2; m=10,000; M=100,000.	Assures production hygiene and microbiological quality of raw materials; absence of enterotoxins; activity of starter culture	Regulation EC 2073/2005 SCA (2015)
Cooking 52-54°C for 45 min	Assures correct moisture levels to control microbial growth; inactivates <i>L. monocytogenes</i> , <i>S. aureus</i> , salmonella and <i>E. coli</i>	Kindstedt (2005) Bachmann and Spahr 1995
Maturation 8-12°C, 85-90% humidity for a minimum of 60 days; average 9 months. Targets: Mean pH of 5.6 (range 5.4-5.8 at end of production; Aw 0.97 and salt content 0.7.	Facilitates reduction of salmonella, <i>E. coli</i> O157:H7 and <i>Listeria</i> during ageing.	Ryser and Marth (1987) Goepfert et al. (1968) D'Amico et al. (2010) Reitsma and Henning (1996)
Process Safety Controls Absence of salmonella/25g cheese at end of production (EOP) and during shelf life Absence of <i>L. monocytogenes</i> / 25g cheese at EOP before the food has left the immediate control of the FBO who produced it or <100 cfu/g during the shelf life Absence of <i>E. coli</i> O157:H7/25g at EOP and during shelf life Enterobacteriaceae <100 cfu/g <i>E. coli</i> <100 cfu/g	Food safety assurance Food safety assurance/hard cheese Food safety assurance Process hygiene Process hygiene and safety	Regulation EC 2073/2005 SCA (2015)

2.3.6. Microbiological Criteria for Cheese

EU microbiological criteria for cheese and milk intended for cheesemaking are risk based and differ depending upon whether cheese has been made from heat treated versus raw milk (Table 31 and 32). In cheese made from heat-treated milk, limits have been established for *Staphylococcus aureus* toxins (food safety criteria), along with targets for *S. aureus* and *E. coli* (process hygiene criteria) (EC 2073/2005) (Table 32).

The application of *E. coli* limits provide a scientifically meaningful standard in cheese made from heat-treated milk as *E. coli* will not survive heat treatment, thus its presence in cheese made from heat-treated milk indicates post-process recontamination. For cheeses made from raw milk, a sampling plan targeting coagulase positive *Staphylococcus aureus* has been established, where $n=5$, $c=2$, $m=10^4$ and $M=10^5$. If three of five samples contain $>10^4$ cfu/g or if values of $>10^5$ cfu/g are detected, the cheese batch has to be tested for staphylococcal enterotoxins. The stage of cheese making where the criterion applies is “at the time during the manufacturing process when the number of staphylococci is expected to be the highest.” For *S. aureus* in soft and semi-soft cheeses, growth occurs primarily in the first cheesemaking phases, from inoculation to salting, so the curd should therefore be tested (Cretenet et al. 2011). Action required in the case of unsatisfactory results includes “improvements in production hygiene and selection of raw materials”.

Table 31: Microbiological Criteria for Cheese in EU Legislation (extracted from Regulation (EC) 2073/2005) in comparison with SCA targets (as per APPENDIX 5.2.2 of SCA Approved Code of Practice (2015)) .

Cheese type	Micro-organisms or toxins	No. of samples	SCA target	Criterion in Regulation (EC) 2073/2005	Stage where the criterion applies
Ready-to-eat foods [cheese] intended for infants and special medical purposes	<i>Listeria monocytogenes</i>	10	Not detected in 25g	Not detected in 25g	Products placed on the market during their shelf-life
Ready-to-eat foods [cheese] able to support the growth of <i>L. monocytogenes</i>	<i>Listeria monocytogenes</i>	5	Not detected in 25g	100 cfu/g ⁸⁵	Products placed on the market during their shelf-life
		5	Not detected in 25g	Not detected in 25g ⁸⁶	Before the food has left the immediate control of the food business operator who has produced it
Ready-to-eat foods [cheese] unable to support the growth of <i>L. monocytogenes</i> ⁸⁷	<i>Listeria monocytogenes</i>	5	Not detected in 25g	100 cfu/g	Products placed on the market during their shelf-life
Cheese [butter and cream] made from raw milk or milk that has undergone a lower heat treatment than pasteurisation ⁸⁸	Salmonella	5	Not detected in 25g	Absence in 25g	Products placed on the market during their shelf-life
Cheese [milk powder and whey powder]	Staphylococcal enterotoxins	5	Not detected in 25g	Absence in 25g	Products placed on the market during their shelf-life

⁸⁵ This criterion applies if the manufacturer is able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 cfu/g throughout the shelf-life. The operator may fix intermediate limits during the process that should be low enough to guarantee that the limit of 100 cfu/g is not exceeded at the end of shelf-life.

⁸⁶ This criterion applies to products before they have left the immediate control of the cheesemaker, when they are not able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 cfu/g throughout shelf-life.

⁸⁷ Products with a pH ≤ 4.4 OR an Aw ≤ 0.92; products with pH ≤ 5.0 AND an Aw ≤ 0.94; products with a shelf-life of < 5 days – shall be considered not to be able to support the growth of *Listeria monocytogenes*.

⁸⁸ Excluding cheeses where the cheesemaker can demonstrate to the satisfaction of the competent authority that due to the ripening time and 'water activity' of the product where appropriate, there is no *Salmonella* risk.

Table 32: Microbiological Criteria for cheese made from unpasteurised or raw milk in EU Legislation (extracted from Regulation (EC) 2073/2005) (as per APPENDIX 5.2.2 of SCA Approved Code of Practice (2015))

Food category	Micro-organisms	Sampling plan		m	M	Stage where criterion applies	Action in case of unsatisfactory results
		n	c				
Cheese made from raw milk	Coagulase-positive staphylococci ⁸⁹	5	2	10 ⁴ cfu/g	10 ⁵ cfu/g	At the time during the manufacturing process when the number of staphylococci is expected to be the highest	Improvements in production hygiene and selection of raw materials. If values > 10 ⁵ cfu/g are detected, the cheese batch has to be tested for staphylococcal enterotoxins. If enterotoxin is detected, then actions as per the Food Safety Criteria shall be taken
Cheeses made from milk that has undergone a lower heat treatment than pasteurisation ⁹⁰	Coagulase-positive staphylococci	5	2	100 cfu/g	1000 cfu/g		

Table: 33. Recommendations for cheese additional to criteria in EU legislation (as per APPENDIX 5.2.3 of SCA Approved Code of Practice (2015))

Product	Test	Criteria in UK HPA Guidelines	SCA Target
All cheese	<i>Escherichia coli</i> O157 and other Shiga toxin-producing <i>E. coli</i>	Unsatisfactory if detected ⁹¹	Not detected in 25g Unsatisfactory if detected
	Enterobacteriaceae	Unsatisfactory if > 10,000 cfu/g (Borderline if 100 -10,000 cfu/g) NB Does not apply to cheese ripened using <i>Hafnia alvei</i> or <i>Proteus vulgaris</i>	< 100 cfu/g
Cheese made from raw milk	<i>Escherichia coli</i> ⁹²	Not specified in UK HPA guidelines	< 100 cfu/g – hard cheese < 10,000 cfu/g – soft and semi-soft cheese

⁸⁹ While EU legislation does not prohibit sale of cheese containing Enterotoxigenic staphylococci at levels exceeding 100,000cfu/g provided that staphylococcal enterotoxin cannot be detected in the cheese, such levels are undesirable. Some regulatory authorities may test the organism to look for the gene that encodes for toxin production and could suggest that presence of this gene demands a product recall even though this is beyond the scope of EU legislation. The gene may be present but it does not automatically follow that enterotoxin is produced. The complex biochemical and microbiological characteristics of cheese can inhibit toxin production despite the multiplication of the organism.

⁹⁰ Excluding cheeses where the manufacturer can demonstrate, to the satisfaction of the competent authorities, that the product does not pose a risk of staphylococcal enterotoxins.

⁹¹ Suggested actions: immediate investigation of the food origin, production process and environment; take food samples and consider environmental monitoring

⁹² Non-toxigenic *Escherichia coli* may sometimes be found in soft, mould ripened or washed-rind cheese made from raw milk. Although regulation (EC) No. 2073/2005 (as amended) has no criteria for *E. coli* in cheese made from raw milk, it is recommended that these cheese types be routinely tested for *E. coli* and an investigation undertaken if a change in trend is detected. A risk assessment is recommended to assess the need for periodic monitoring for STEC O157. Tests should be undertaken urgently where there is epidemiological evidence linking STEC infection with a specific food.

The Health Protection Agency (HPA), now Public Health England (PHE) has produced [guidelines](#) for the assessment of the safety of RTE foods marketed in the UK. As they note, the only food safety criterion for staphylococci is an absence of SEs in cheese, milk powder and whey powder during shelf life. They recommend however, that should cheese products sampled at retail have $>10^3$ cfu/g coagulase positive staphylococci then further investigation should be conducted to determine the cause of the high counts. They further recommend that for samples with levels $>10^4$ cfu/g, any isolated strains should be tested for enterotoxin genes. Cheesemakers should be aware of these recommendations and strive to achieve $<1,000$ cfu/g of coagulase positive staphylococci in cheese.

In establishing EU Community microbiological criteria, the EU believes that such criteria should: enhance food safety, be feasible in practice and be based on scientific risk assessment. ICMSF (The International Commission on Microbiological Specifications for Foods) guidance was considered in establishment of microbiological criteria for cheese in EC Regulation 2073/2005. ICMSF is recognised as the global leading scientific body for establishment of microbiological criteria in foods. ICMSF provides global guidance for sampling plans for foods. In ICMSF Book 2, in its risk assessment for cheese, ICMSF state: *"While the coliform problem in cheese is well known, presence of these organisms in many cheese varieties is extremely difficult to prevent completely. With some varieties, if coliforms are present initially, it is virtually impossible to prevent their growth during manufacture or during the ripening period. In several types of cheese E. coli can even be considered characteristic. With the exception of some strains of E. coli high populations of coliforms are unlikely to present a health hazard. There is ample evidence that if pathogenic strains of E. coli (PEC) are present early in the cheesemaking process their numbers may increase to hazardous levels. However, in view of the scarcity of evidence of recurring outbreaks due to PEC in cheese and the high cost of routine testing, it is doubtful that establishment of end-product criteria for either coliforms or E. coli would be justified. Accordingly, no sampling plan is proposed."*

In ICMSF Book 8 (2011), Table 23.7 outlines end-product testing criteria for cheeses. In cheeses made from pasteurised milk, *E. coli* limits are established under a sampling plan where $n=5$, $c=3$, $m=10$ and $M=10^2$. Raw milk cheese is tested for *Staphylococcus aureus* only, consistent with EU recommended sampling criteria. It is notable that for EU microbiological criteria for cheese, no limits were established for *E. coli* in raw milk cheese. *E. coli* does not offer a meaningful hygienic index in raw milk cheese as its presence is expected, consistent with guidance from ICMSF. However, the HPA recommends that raw milk cheese be tested routinely for *E. coli*, and if detected, the source of contamination investigated, particularly if an upward trend is noted since STEC may be present. For ensuring raw milk safety, ICMSF recommends that raw milk cheesemakers establish a good supplier relationship for critical ingredients (raw milk) and target the absence of salmonella, EHEC and *L. monocytogenes*.

The draft UK policy position considers the presence of STEC in RTE food a serious risk to public health⁹³ (Table 34). FSS/FSA considers the presence of STEC in food confirmed when the presence of *stx* genes are detected in an isolated *E coli* strain. In this case, FSS/FSA requires that the Competent Authority be notified through incident reporting procedures and the affected product batches withdrawn from the market.

⁹³ https://www.food.gov.uk/sites/default/files/enf-w-16-016-draft_uk_working_policy.pdf

Table 34: Adapted from draft FSA/FSS UK policy position: Summary of action required in response to unsatisfactory test results from both OC and FBO sampling and testing

	Presumptive test result	Confirmed test result
Definition	Detection of one or more <i>stx</i> gene(s) is considered a presumptive positive result if their presence has not been confirmed in an isolated <i>E. coli</i> strain	Presence of STEC is confirmed when one or more <i>stx</i> gene(s) are detected in an isolated <i>E.coli</i> strain.
Food Profile 1	Action required by Local Authority (LA)/FBO	Action required by LA/FBO
RTE foods; Foods to be consumed with a mild heat treatment unlikely to remove the STEC risk And Food for which an FBO is not able to provide guarantees that a treatment that will remove the STEC risk will be applied	<p>The Competent Authority should be notified through incident reporting procedures. FBOs should prepare to take immediate action in readiness for a confirmed test result:</p> <ul style="list-style-type: none"> • Retain affected batch(s) still within FBO control; • Collate traceability information; • Notify onward supply chain and request retention of affected product still within their control. <p>If presumptive results are not confirmed, held product can be released on to the market with no further action.</p> <p>There may be instances where a withdrawal or recall may be required by the competent authorities on the basis of detection of <i>stx</i> genes alone, if, for example, there is epidemiological or other information that links the food to cases of illness.</p>	<p>The Competent Authority should be notified through incident reporting procedures.</p> <p>The affected batch(s) of food must be withdrawn from the market in accordance with Regulation (EC) 178/2002. Information on onward supply of the product will be required to determine whether a product recall from end users/ consumers is required.</p> <p>Investigations should be initiated by the FBO to identify and eliminate the source of STEC contamination and any other batches or products affected.</p> <p>The FBO should review the HACCP-based food safety management system, to ensure that STEC is identified as a specific hazard and that the risk from STEC in food is minimised.</p>
	Presumptive test result	Confirmed test result
Definition	Detection of certain genes* confirmed in an isolated <i>E. coli</i> strain. 12 associated	Presence of certain STEC strains associated with severe disease is confirmed i.e. when specific genes* are

	with severe disease is considered a presumptive positive result if their presence has not been confirmed in an isolated <i>E. coli</i> strain.	detected in an isolated <i>E. coli</i> strain.
Food Profile 2	Action required by LA/FBO	Action required by LA/FBO
Raw foods or foods intended to be consumed following a treatment that will remove the STEC risk	<p>It is reasonable to wait for the completion of confirmatory tests before action is taken.</p> <p>FBOs may, according to their own risk assessments or commercial operations, take risk management action on the basis of a presumptive positive result.</p>	<p>Confirm the affected batch(s) is labelled or accompanied by appropriate cooking and handling instructions to ensure it will be treated or cooked sufficient to remove the STEC risk OR Re-label the affected batch(s) retrospectively or ensure it is accompanied by appropriate cooking and handling instructions as above. OR Product still within the FBOs control can be redirected to an alternative use e.g. further processing sufficient to remove the STEC risk; OR Provide evidence that the product will be further processed sufficient to remove the STEC risk. If none of the above actions are taken, the affected batch(s) must be withdrawn from the market. Investigations should be initiated by the FBO to identify and eliminate the source of STEC contamination and any other products affected. The FBO should review the HACCP-based food safety management system, to ensure that STEC is identified as a specific hazard and that effective and proportionate controls are in place to minimise the risk from STEC.</p>

* genes for one of the top six STEC serogroups most frequently associated with serious human illness in Europe (O157, O26, O103, O145, O111, O104) in combination with stx and [1] eae or [2] aaiC and aggR genes

2.3.7. On site and laboratory testing for microorganisms

A number of dehydrated and chromogenic testing formats are available to facilitate the ease with which cheesemakers can conduct their own on-premise safety evaluations. On site testing by cheesemakers for *Listeria*, salmonella or STEC is, however, not recommended. Analysis for these pathogens should be performed by an accredited laboratory.

Generic (non-toxigenic *E. coli*) and coagulase-positive staphylococci evaluation can be done on site using systems such as Petrifilm™. The Petrifilm™ Staph Express Count Plate is a sample-ready culture medium system that contains a water-soluble gelling agent. The chromogenic medium (modified Baird-Parker) is both selective and differential for *Staphylococcus aureus*, *S. hyicus* and *S. intermedius*. DNase-positive organisms detected on the Petrifilm™ Staph Express plate are *S. aureus*, *S. hyicus* and *S. intermedius*. These three microorganisms represent the majority of coagulase-positive staphylococci CPS⁹⁴. Initial validations of this method (2003) were conducted in accordance to the EN ISO 6888-1: 1999⁹⁵ standard: Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) – Part 1: Technique using Baird-Parker agar medium. Viçosa et al. (2010) evaluated cultural media and methods including Baird Parker Agar, Rabbit Plasma Fibrinogen agar (RPFA) and the Petrifilm™ Staph Express count system (STX) for enumeration of coagulase and thermonuclease positive *Staphylococcus* species in raw milk and fresh soft cheese. No differences in the mean count were observed for these media, although RPFA and STX showed good correlation between total and typical colony counts as well as coagulase and thermonuclease-positive colony counts.

Jasson et al. (2009) found that tryptic soy broth (TSB) failed to recover injured *E. coli* O157 from foods compared to buffered peptone water (BPW) and cautioned that use of TSB for enrichment could lead to false negative results. Marozzi et al. (2016) analysed two standard methods for STEC detection: a cultural method (ISO 16654:2001⁹⁶) and a molecular method (ISO 13136:2012⁹⁷). These authors were able to confirm only two *E. coli* O157:H7 strains using the cultural procedure, and neither was *stx1*, *stx2* or *eae* positive. In comparison, the molecular method revealed 22 *stx*-positive samples, with results showing a higher prevalence of virulence-associated genes in dairy products made from raw sheep milk.

Voysey et al. (2012) reported success in detection of STEC through use of modified TSB for enrichment at 41.5°C, followed by immunomagnetic separation and streaking to chromogenic media (ChromID O157 agar (for O157 and O26). XP CEN ISO/TS 13136 is the horizontal method for detecting STEC and the determination of O157, O111, O26, O103 and O145 serogroups (EFSA 2013). Delannoy et al. (2016) used five novel markers to reduce the number of false positive results by 48% and improved the discriminatory power of EHEC screening consistent with EFSA opinions.

Enterobacteriaceae (EB) testing is useful for assessing the hygiene status of foods. Their presence in heat-treated foods signifies post-process recontamination. The SCA recommends that cheeses be tested for EB and levels should be <100 cfu/g for hard cheese, <10,000 cfu/g for mould-ripened soft and semi-soft cheese and <100,000 cfu/g

⁹⁴ https://nf-validation.afnor.org/en/wp-content/uploads/sites/2/2014/03/Synt-3M-01-09-04-03-B_en.pdf

⁹⁵ <https://www.iso.org/standard/23036.html>

⁹⁶ <https://www.iso.org/obp/ui/#iso:std:iso:16654:ed-1:v1:en>

⁹⁷ <https://www.iso.org/standard/53328.html>

for washed rind soft and semi-soft cheese. Coliform testing is frequently performed to assess whether cheeses have been manufactured under sanitary conditions, but EB testing provides a more conservative assessment of hygiene. Trmčić et al. (2016) cautioned that generic coliform testing cannot be used to assess the safety of natural cheese. These authors recommended that coliform testing be replaced by testing for generic *E. coli*, a better indicator of faecal contamination; and testing for *L. monocytogenes*. Results of this review are in agreement with this recommendation.

2.3.8. Measurement of physicochemical parameters

Ensuring that cheesemaking achieves desired and consistent physicochemical parameters is an essential part of food safety assurance. The physicochemical parameters that impact microbial growth and survival include:

- acidity (measured through pH or titratable acidity),
- moisture content (measured through water activity (A_w), percent moisture, or moisture on a fat-free basis MFFB); and
- salt content (expressed most meaningfully as % salt-in-moisture).

When these factors are consistent, there is predictability in the microbiological behaviour and therefore safety of the produced cheese. Conversely, variations in these parameters often lead to microbiological defects and can compromise cheese safety (Trmčić et al. 2016; Trmčić et al. 2017) as well as compromising the quality of the cheese. In general, low moisture, low pH cheeses (such as Cheddar and Italian Grana) are microbiologically stable compared to cheeses with high moisture and more neutral pH values (such as Camembert and Brie) (Choi et al. 2016).

Physicochemical parameters can be useful in assessing risk of specific pathogens in cheese. For example, Camembert and Feta are identical in composition for percent moisture, water activity, % salt-in-water and ripening temperature. However, fully ripened Camembert has a pH of 7.5 compared to Feta with a pH of 4.4 which inhibits growth of *L. monocytogenes* (Donnelly 2004). In cheeses where pH and A_w characteristics allow the growth of pathogens, refrigerated storage temperature may be used as a controlling parameter (Araújo et al. 2017).

Kindstedt (2005) recommends that cheesemakers establish two critical parameters: 1. an optimum acidity schedule and 2. initial composition targets (pH, A_w , % salt). The author emphasises the need for cheesemakers to understand the correct compositional targets for their cheese, as well as the need for measurement to ensure that those targets are consistently met. Correct and controlled acidity development is crucial to quality cheese production as well as for growth, inhibition and/or minimising survival of pathogenic and spoilage flora in cheese (SCA, 2015). Kindstedt (2005) recommends acidity measurements at key stages during cheesemaking. For Cheddar cheese, the 5 key stages include: starting milk pH; milk pH immediately after addition of starter culture; the whey pH immediately after curd cutting; the whey pH at the beginning of

draining; and the whey or curd pH at milling. For bloomy rind, blue mould smear-ripened and washed rind cheeses, the pH or the curd or whey flowing from the draining racks should be measured at specified times during draining and moulding. The final pH measurement should be taken of cheese the following day. For Swiss and hard Italian style cheeses that are pressed after draining or dipping, the final pH measurement should be taken the day after completion of pressing (Kindstedt, 2005). The SCA (2015) indicates the need for an optimum acidity profile for each unique cheese type produced, along with a description of corrective action that will happen if deviations from this profile occur.

Initial cheese composition targets (pH, A_w , salt-in-moisture) should be measured on a specified date, for example, 7 days post-manufacture. The SCA (2015) provides advice on what the cheesemaker should consider when designing their sampling plan, to ensure that samples are representative. Bradley and Vanderwarn (2001) warn that incorrect sampling may introduce significant errors in the results. These authors recommend use of a cheese trier to pull three representative plugs from the cheese, with one from the center, one from the outer edge and one in-between the center and outer edge. The three representative plugs should be blended using a commercial blender to yield a single representative sample.

The Codex Alimentarius Commission classifies cheese types according to firmness based upon moisture on a fat-free basis (see Table 1). This is determined by the weight of moisture in the cheese/total weight of cheese - weight of fat in the cheese x 100. Cheeses with MFFB of <51% are categorised as extra hard, those with MFFB values between 49-56% as hard, cheeses with MFFB of 54-63% as semi-hard, semi-soft cheeses have MFFB values of 61-69% and soft cheeses have MFFB values >67%. As can be observed in these values, there is overlap in MFFB between categories, so cheeses such as Cheddar may be described as hard/semi hard, for example, dependent on how long the cheese has been aged for.

$\text{MFFB} = \frac{\text{Weight of moisture in cheese}}{\text{Total weight of cheese} - \text{weight of fat in cheese}} \times 100$

2.3.8.1. Measuring Acidity

Kindstedt (2005) notes that due to the importance of acid production by the starter culture used in cheesemaking to the quality and safety of cheese, it is essential for cheesemakers to routinely monitor acidity during cheesemaking. This can be done by measuring titratable acidity or pH. Titratable acidity (TA) measures all acid molecules in a sample, whereas pH measures free hydrogen ions in water. Kindstedt has outlined methods that can be used by cheesemakers in measuring acidity (pH or TA) during cheesemaking. He cautions that while TA works well for liquid samples, it is not easily

adapted to solid curd measurement. TA would be less useful with cheese that develop the majority of their acidity during pressing (Swiss-style cheeses, for example). Measuring acidity development using pH is frequently used by cheesemakers because pH measurements can be easily obtained from both liquid and solid samples (Kindstedt 2005).

Portable, handheld cheese pH meters are readily available to artisan cheesemakers. It is important that pH meters are calibrated with standardised buffer solutions each day prior to use.

2.3.8.2. Measuring A_w

Water activity (A_w) is a measure of the “free” water in a system that may be utilised by any microorganisms present (i.e. not the water which is bound to other molecules and therefore “unavailable”). It therefore serves as a better indicator of the safety/ stability of cheeses than total water content and can be measured at the end of production or the end of maturation, if changes are expected during maturation. A_w in cheese is most easily determined with a calibrated water activity meter (Ferrier et al. 2013; Banks 2006).

2.3.8.3. Measuring Salt

Salt in cheese is easily measured using a chloride specific ion meter (Kindstedt and Kosikowski 1984; D'Amico et al 2014; Johnson and Olson 1985) or titrator strips for chloride. For use of test strips (Kindstedt 2005), the bottom of the test strip is placed into a water extract of a cheese sample (see below), allowing chloride ions to diffuse and react with silver ions in the test strip, forming silver chloride. A colour change occurs in the test strip with colour proportional to the concentration of chloride ions. To make a water extract of cheese, finely ground cheese (10 grams) is placed in a blender jar and combined with 90 ml of boiling water. The mixture is blended at high speed for 30 seconds to extract the chloride from the cheese. The water mixture is cooled, and then qualitative grade filter paper is folded into a cone and placed into the water extract. The test strip is placed into the water that collects at the bottom of the filter cone, and the reading on the titrator scale is converted to % NaCl, multiplying by 10 to account for the initial sample dilution with water.

2.3.8.4. Measuring Moisture

Kindstedt (2005) and Bradley and Vanderwarn (2001) describe methods for moisture determination in cheese. Moisture testing instruments based on infrared or halogen drying provide rapid results and are relatively inexpensive. Instruments based on microwave drying or near-infrared absorption are also available but are more costly. Traditional oven drying is the method most practical for artisan cheesemakers and that method is summarised briefly here.

The required equipment consists of a laboratory oven operating at 100°C, disposable aluminum weighing dishes, a digital electronic balance and a desiccator containing a

desiccant (calcium sulphate is preferred). A cheese sample should be ground then analysed within 24 h. A blender works well for purposes of grinding cheese samples. The sample should be weighed into disposable aluminum dishes that have been pre-dried for 3 h at 100°C before use. The dishes should be stored in a desiccator with active desiccant prior to use. Moisture determination is performed in duplicate. 2-3 grams of finely ground cheese are added to two weighed empty aluminum dishes, and the weight of the empty dish is subtracted to obtain the weight of the cheese sample. The dishes are placed in the 100°C oven for 24 \pm 1 hour, then reweighed. The total solids and moisture contents are determined as follows:

$$\% \text{ total solids} = \frac{\text{dry weight of cheese}}{\text{Wet weight of cheese}} \times 100$$

$$\% \text{ moisture} = 100 - \% \text{ total solids}$$

2.3.7.5 Measuring Salt in Moisture

It is important for cheesemakers to monitor this parameter, however many do not. The calculation below is one of the ways that salt in moisture can be calculated:

$$\text{Salt in moisture} = (\% \text{ salt in cheese} \div \% \text{ moisture in cheese}) \times 100\% \text{ (Kindstedt, 2005)}$$

ACMSF provides an additional method of calculating the % water phase salt content (WPS)⁹⁸. In addition to pH/titratable acidity measurements, salt-in-moisture measurements provide evidence of process control during cheesemaking. The cheeses depicted in the figures below (Figures 10 and 11) were from a study of Cheddar cheese produced from cows' milk by a farmstead cheesemaker opting to switch from year-round to seasonal production. These cheeses were higher in moisture and lower in salt than ranges optimal for Cheddar production. By weekly monitoring of salt-in-moisture data, it would have been evident to the cheesemaker to increase salt addition to the curd, allowing production for a cheese better suited to long ageing, with resultant assurance of microbiological safety.

⁹⁸ Guidance Note No. 18 Validation of Product Shelf-life (Revision 3) Published by: Food Safety Authority of Ireland ISBN 1-904465-33-1

Figure 10: Seasonal Variation in Moisture Content of Farmhouse Cheddar (adapted from Kindstedt, 2005)

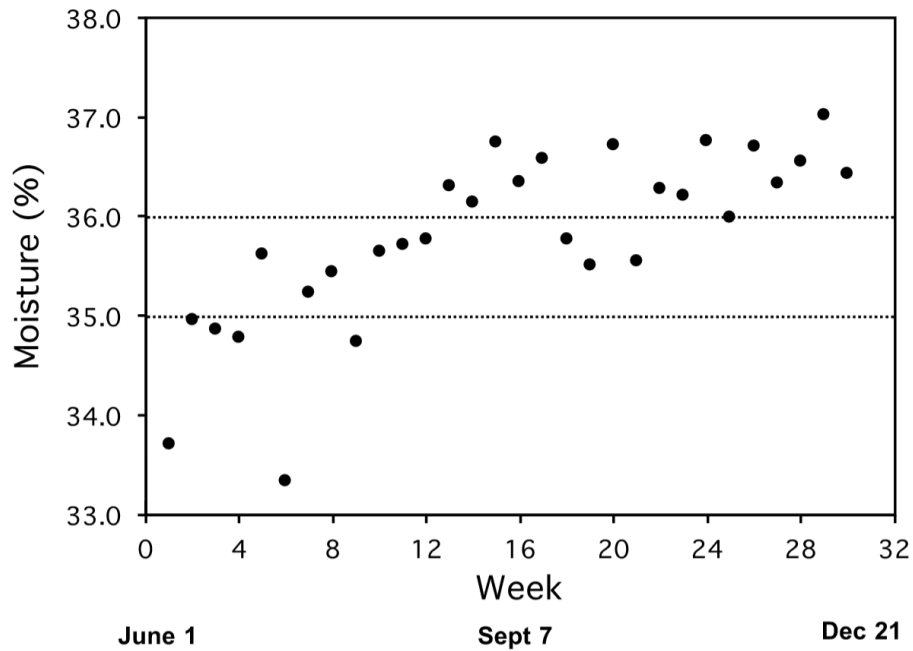
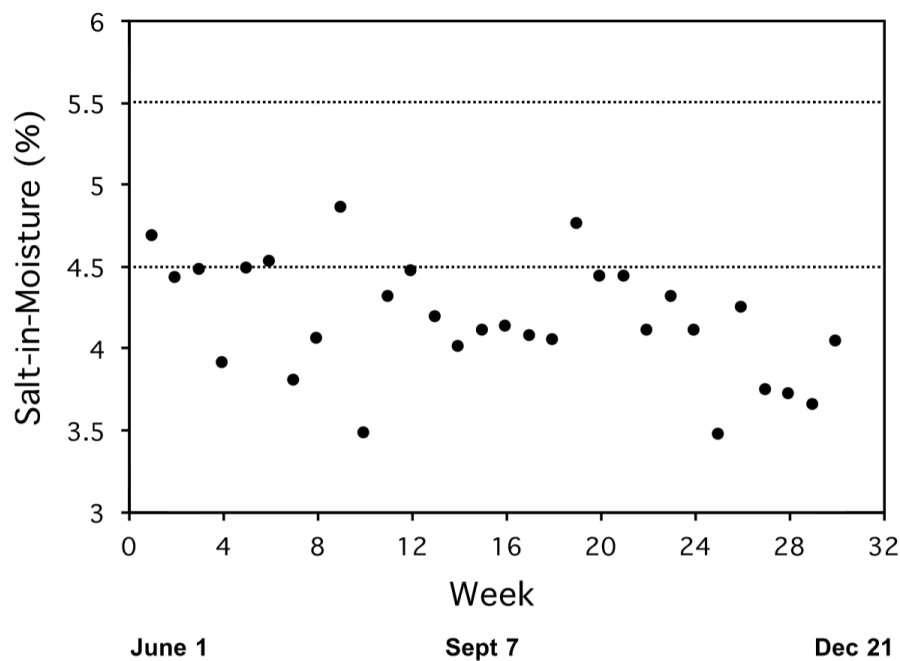


Figure 11: Seasonal Variation in Salt-in-Moisture Content of Farmhouse Cheddar (adapted from Kindstedt, 2005)



The SCA recently used pH, A_w and moisture on a fat free basis (MFFB) values for UK cheeses to assign risk rankings for cheeses based upon their requirement for storage at

temperatures of $\leq 8^{\circ}\text{C}$ during shelf life. Risk rankings of 1 (low concern) to 9 (high concern) were assigned to cheeses (Figure 12). It was deemed that cheese varieties with an overall risk ranking of 4 or lower should be classified as low risk and do not require maturation or storage at temperatures of $\leq 8^{\circ}\text{C}$. Those cheeses with risk rankings of >4 may require storage at temperatures $< 8^{\circ}\text{C}$ during shelf life.

Table 35: SCA General Risk Assessment for select cheeses based on salt, moisture and pH values⁹⁹. This is a general microbiological risk assessment. It is intended to be an overview that identifies which cheeses, in general terms, to be 'microbiologically robust' and which might be considered 'microbiologically sensitive'

CHEESE VARIETY	MFFB (%)	SALT-IN-MOISTURE:		pH IN SHELF LIFE:		OVERALL RISK RATING	LEVEL OF CONCERN
		Value (%)	Risk rating	pH range	Risk rating		
Parmesan, 'fresh'	39.3	5.7	1	5.2-5.4	2	2	Low
Emmental	51.1	1.3	3	5.5-6.0	3	9	High
Gruyère	52.5	4.6	1	5.5-5.8	3	3	Low
Cheddar, English	56.2	4.8	1	5.0-5.6	2	2	Low
Red Windsor	56.3	4.5	1	5.2-5.8	3	3	Low
Red Leicester	56.5	4.3	1	5.2-5.8	3	3	Low
Hard cheese, average	56.9	4.5	1	5.2-5.6	2	2	Low
Derby	57.5	3.8	1	5.2-5.8	3	3	Low
Sage Derby	57.5	3.9	1	5.2-5.8	3	3	Low
Gouda	58.2	5.5	1	5.1-5.6	2	2	Low
Double Gloucester	58.3	4.3	1	5.1-5.6	2	2	Low
Stilton, blue	58.5	5.0	1	5.5-6.5	3	3	Low
Edam	59.2	5.5	1	5.2-5.6	2	2	Low
White Cheshire	59.7	3.0	2	5.0-5.5	2	4	Medium
Lancashire	60.2	3.4	2	5.0-5.5	2	4	Medium
White cheese, average	60.3	3.0	2	5.0-5.5	2	4	Medium
Caerphilly	60.8	2.8	2	5.0-5.5	2	4	Medium
Wensleydale	61.0	2.6	2	5.0-5.5	2	4	Medium
Roquefort	61.5	9.3	1	5.5-6.5	3	3	Low
Paneer	62.0	0.1	3	5.4-5.6	2	6	Medium
Port Salut, St Paulin	62.9	3.7	1	5.2-5.4	2	2	Low
Danish blue	65.1	6.3	1	5.2-6.4	3	3	Low
Halloumi	66.5	5.7	1	5.9-6.4	3	3	Low
Dolcelatte, rind removed	66.7	5.1	1	5.5-6.5	3	3	Low
Stilton, white	66.7	4.1	1	5.0-5.5	2	2	Low
Lactic, goats' cheese	68.5	2.9	2	4.1-4.6	1	2	Low
Brie, coat removed	68.7	2.8	2	5.5-7.0	3	6	Medium
Camembert	70.4	2.8	2	5.5-7.0	3	6	Medium
Feta	70.8	4.3	1	4.2-4.8	1	1	Low
Mozzarella, 'fresh'	72.0	1.7	3	5.0-5.5	2	6	Medium
Ricotta	81.0	0.4	3	5.6-6.2	3	9	High
Mascarpone	83.2	0.4	3	5.8-6.2	3	9	High

⁹⁹ SCA ACOP CODICIL 1: Part 2, 2017 <http://www.specialistcheesemakers.co.uk/article/extended-assured-advice-2017-21.aspx>

2.4. Summary of recommendations from Chapter 1

Codex has historically classified cheese according to firmness, fat content and principal curing characteristics. The growing consumer interest in artisan cheese consumption is driving production of a diversity of cheese styles, which complicates cheese classification for safety assessment. Cheese classification based on pH and Aw is more useful (in relation to Codex classification) for the assessment of risk of survival of STEC, *L. monocytogenes* and other pathogens, but alternative classification schemes that account for processing steps where pathogens can be introduced or reduced should also be considered.

The microbiological safety of cheese made from unpasteurised milk is principally dictated by the microbiological quality of raw milk used for its production. Improving milk hygiene is the most significant factor leading to the safety of cheeses made from raw milk¹⁰⁰ (FSAI 2015; Jaakkonen et al. 2017; Doyle et al. 2017; Farrokh et al. 2013). This can be accomplished through herd management, mastitis control, a focus on feeding regimes and overall sanitation during milking, storage and transportation to the cheesemaker. Monitoring the effectiveness of these strategies can be accomplished through regular testing and monitoring to assure that microbiological criteria are met. When criteria are exceeded, it is critical that cheesemakers determine the cause for elevated microbiological counts and bring levels back into acceptable ranges.

The most significant pathogens of concern to the safety of cheeses made from unpasteurised milk are STEC, *S. aureus*, *L. monocytogenes* and salmonella. For some cheeses, the cheesemaking process will not reduce levels of these pathogens; therefore, it is critical that the milk used for cheesemaking be of high microbiological quality.

The primary defence for preventing STEC contamination of cheese is milking hygiene and prevention of faecal contamination of milk. Even when STEC are present in cow faeces, appropriate sanitary practices lower the risk of milk contamination (Lambertini et al. 2015). Considering the wide on-farm distribution of STEC, educational programming should be conducted to bring about awareness of STEC issues among farmers, cheesemakers and consumers. Research is needed to identify and eliminate vehicles introducing STEC to dairy cattle in order to reduce on-farm prevalence and improve raw milk cheese safety.

Microbiological contaminants in the dairy processing environment are important sources of finished product contamination. Risk reduction efforts should be placed on the identification of reservoirs of pathogens such as *Listeria monocytogenes* in the production system and the development of practices that reduce pathogen spread and minimise the potential for cheese contamination. Effective environmental monitoring and

¹⁰⁰<https://www.foodstandards.gov.au/code/proposals/documents/P1007%20PPPS%20for%20raw%20milk%201AR%20SD3%20Cheese%20Risk%20Assessment.pdf>

elimination of *Listeria* spp. within processing plants, including farmstead cheese operations, is a key component of a *Listeria* control program.

HACCP and control of hazards through identification of CCPs is a legal requirement in the UK and EU. Monitoring incoming milk temperature and storage temperature, establishing a target acidity schedule, testing for coagulase positive staphylococci as a process safety control, proper salting and achieving desired physicochemical targets are all examples of controls that impact microbiological safety.

Codex¹⁰¹ issued “Guidelines for validation of food safety control measures.” Codex recognises five approaches for validation, and one is to reference scientific literature, an approach taken in this report. Another is use of mathematical modeling and use of statistically valid surveys, and this has also been done in the report. Many of the steps in raw milk cheesemaking are not CCPs but rather preventive controls. In the U.S., published literature can be used to validate a preventive control, consistent with Codex recommendations. Cheesemakers must monitor CCPs each time a cheese is made to ensure that the cheesemaking process is controlling identified hazards. Microbiological testing verifies that the HACCP plan is working as intended. During epidemiological investigations of outbreaks involving cheese, collection of compositional data (pH, salt and moisture) could reveal important information about causative factors, including lack of process control.

¹⁰¹http://webcache.googleusercontent.com/search?q=cache:u3ah_z03kdYJ:www.fao.org/input/download/standards/11022/CXG_069e.pdf+&cd=1&hl=en&ct=clnk&gl=us

Chapter 2: An analysis of currently available predictive modeling and challenge testing methods that are applicable to cheesemakers

3.1 Predictive Models

A number of predictive models are available to assist cheesemakers in making safety decisions concerning their products. These include ComBase¹⁰², The Food Spoilage and Safety Predictor¹⁰³, the USDA Pathogen Modeling Program¹⁰⁴ and the Raw Milk Cheese Decision Support Tool¹⁰⁵. In general, the majority of predictive models developed to date have been shown to have limited value in predicting the fate of microbial pathogens in cheese for reasons subsequently detailed (Araújo et al. 2017; Schwartzman et al. 2010; Schwartzman et al. 2014a; Kocharunchitt 2015). An exception may be the Raw Milk Cheese Decision Support Tool, discussed below.

Most predictive models are based upon growth of a singular pathogen in a defined broth (laboratory) based system where the growth or decline of a pathogen of interest is followed over time. These conditions are unlikely to replicate what a pathogen encounters when growing in a cheese. Instead of growth as a singular population, pathogens in cheese grow or compete in complex microbial communities comprised of diverse bacterial and fungal genera (Wolfe et al. 2014). In addition, the microbial community exhibits dynamic changes as the cheese ages and matures. In a static broth system, such population dynamics do not exist. Models such as the Food Spoilage and Safety Predictor and the Raw Milk Cheese Decision Support Tool were specifically developed to overcome these challenges.

3.1.1 The USDA Pathogen Modeling Program (PMP)

The U.S. Department of Agriculture (USDA) Pathogen Modeling Program (PMP) is a predictive microbiology tool designed primarily for research and instruction in estimating the effects of multiple variables on the growth, inactivation, or survival of foodborne pathogens. A key limitation is that the majority of the developed models in the PMP are based on experimental data of microbial behavior in liquid microbiological media. Because of this, the PMP is not likely to provide cheesemakers with accurate results regarding the fate of a pathogen in cheese and may predict pathogen growth when it is unlikely to occur.

3.1.2 ComBase

ComBase has similar limitations to the USDA PMP. It is the result of a collaborative effort between the University of Tasmania and the USDA Agricultural Research Service. ComBase predictive models consist of a set of twenty growth models, seven thermal

¹⁰² <https://www.combase.cc/index.php/en>

¹⁰³ <http://fssp.food.dtu.dk/>

¹⁰⁴ <https://www.ars.usda.gov/northeast-area/wyndmoor-pa/eastern-regional-research-center/residue-chemistry-and-predictive-microbiology-research/docs/pathogen-modeling-program/pathogen-modeling-program-models/>

¹⁰⁵ <http://www.foodsafetycentre.com.au/RMCtool.php>

death models and two non-thermal survival models. Temperature, pH and A_w (primarily as a function of NaCl) are the principal factors used to predict the fate of pathogens in foods. In addition, for some organisms, the effect of a fourth factor (such as CO₂, nitrite, etc.) is also included. ComBase utilises growth kinetics calculated from bacterial growth in laboratory media as opposed to food matrices. As such, this model does not consider specific parameters of food that could impact microbial growth, such as the solid structure of cheese, oxygen diffusion in cheese, background microflora, presence of enzymes, peptides, organic acids and other components that affect bacterial growth and survival in cheese (Araújo et al. 2017).

In studies of growth of *L. monocytogenes* and *S. aureus* in Coalho (a Brazilian cheese), Araújo et al. (2017) found smaller maximum growth values compared to Gr_{max} (maximum growth rate) predicted by ComBase. The authors postulated that high background levels of *Lactococcus* (7.51-8.22 log cfu/g) and *Lactobacillus* spp. (7.33-7.95 log cfu/g) and their associated metabolic products (for example because lactic acid will lower the pH and decrease the growth of most pathogens) accounted for the difference in growth rates observed in cheese versus those predicted by ComBase (Araújo et al. 2017). Schwartzman et al. (2010) concluded that use of models evaluated in tryptic soy broth or even in milk could not be used for predictions of *L. monocytogenes* behaviour in cheese, and argued for the need for generation of real food models.

In follow-on studies, these authors (Schvartzman et al. 2014b) used ComBase as a predictive microbiology tool to estimate the growth potential for *L. monocytogenes* in soft and smear ripened cheese. These authors found in 40% of the cases where ComBase predicted *L. monocytogenes* growth, there was no actual growth of *L. monocytogenes* in cheese when determined experimentally using cheeses spiked with *L. monocytogenes*. Dalmaso and Jordan (2013) examined the growth of *L. monocytogenes* in naturally contaminated Irish farmhouse cheese. The low pH values observed (pH 5.5) and the salt: moisture (S:M) ratio of greater than 6% likely explained the absence of *L. monocytogenes* growth during ripening. Although the authors failed to observe growth of *L. monocytogenes* in two different and independent batches of naturally contaminated (<20 cfu/g) Cheddar cheese, ComBase predicted growth reaching 100 cfu/g after 4 days, and 10⁸ cfu/g following 5 months of ripening from beginning values of <10 cfu/g. The absence of growth observed by the authors was attributed to the pH values observed (pH 5.5) and the S:M ratio of greater than 6%, along with the presence of competitive flora including lactic starter cultures. In conclusion, ComBase is likely to over-predict pathogen growth and survival in cheese and thus may have limited applications for cheesemakers.

3.1.3 Tertiary Predictive Model

Rosshaug et al. (2012) developed a tertiary predictive model to assess the growth of *L. monocytogenes* in a soft blue-white cheese as a function of temperature, pH, NaCl and lactic acid. The model was based on broth data produced from previous studies, and

thus may have the same inherent limitations. The authors found that numbers of *L. monocytogenes* could increase by 3 to 3.5 log within the shelf life of the cheese and further state that this exceeds the limit (<2 log increase) needed to fulfill the EU's food safety criteria (Regulation EC 2073/2005) for cheese where <100 cfu/g of *L. monocytogenes* must be maintained throughout the product shelf life. The authors concluded that prevention of post-pasteurisation contamination was essential to meet food safety criteria for soft blue-white (Danish Blue) cheese. For cheesemakers producing similar cheeses, this model may supply useful documentation regarding fate of *Listeria* and expected growth levels during shelf life.

3.1.4 Other Models

Kocharunchitt (2015) evaluated a variety of predictive models for evaluation of pathogen growth potential in cheeses. For example, they examined the Augustin model (2005) was developed using 2,724 growth/no-growth data sets, with 1,980 obtained in microbiological media from 39 studies, 196 in liquid dairy products from 15 studies, 144 obtained in cheeses from five studies, 324 obtained in meat products from 17 studies and 80 obtained in seafood products from six studies. This model was found to have a poor ability to discriminate growth-permissive versus growth-preventing conditions in cheese. The authors offer two likely factors to explain why some strains of *L. monocytogenes* display slower growth in cheese than growth predicted by the liquid-based generic models. The first is the physical structure of cheese and the antimicrobial bacteriocin nisin produced by the lactic acid bacteria in the cheese, which may inhibit the growth of *L. monocytogenes*. Neither of these factors is included as a controlling parameter in generic predictive models, offering an explanation as to why generic models can overestimate *L. monocytogenes* growth in cheese. In addition, the significant variation in maximum growth rates between different strains in the same soft blue-white cheese matrix highlights the importance of challenge testing a variety of strains that have been isolated from cheese and are therefore adapted for growth in cheese.

Similarly, the Schwartzman model (Schvartzman et al. 2010) had limited applicability due to its lack of consideration of dynamic changes during cheese ageing (changes in the composition of the complex microbial community, production of a variety of metabolic products due to growth (including bacteriocins), death of members of the microbial community and changes in pH, A_w etc.), the limited range of predictor variables and the consideration of a single temperature.

The Mejlholm and Dalgaard (2009) model, discounted by Kocharunchitt (2015) because of lack of available lactic and acetic acid concentration data, was found to have a high proportion of correct predictions of growth/no growth of *Listeria* in a variety of cheeses. The authors indicate that Codex (and Regulation EC 2073/2005) considers that foods with pH 4.4, A_w <0.92 or foods with both pH <5 and A_w <0.94 prevent the growth of *Listeria*. However, many cheeses not meeting these criteria do not support *Listeria* growth, indicating the involvement of other physicochemical parameters that impact

growth conditions. It is widely recognised that growth limiting parameters interact to reduce the growth permissive range of a single physicochemical factor (the hurdle effect).

3.1.5 The Food Spoilage and Safety Predictor (FSSP)

The National Food Institute of the Technical University of Denmark developed The Food Spoilage and Safety Predictor (FSSP). This program contains models to predict the effects of food product characteristics on the growth of both spoilage and pathogenic bacteria in foods. In addition, the software can predict the impact of both constant as well as fluctuating food storage temperatures on food product shelf life and safety.

Recently, an extensive model was developed for the FSSP to predict the growth of *Listeria monocytogenes* in cottage cheese (Ostergaard, Eklow, and Dalgaard 2014). The models developed by the authors were validated using 25 growth rates for *L. monocytogenes*, 17 growth rates for lactic acid bacteria, and 26 growth curves with simultaneous growth of lactic acid bacteria and *L. monocytogenes* in cottage cheese. This model can be used to make growth predictions for *L. monocytogenes* in cottage cheese when stored under refrigeration at constant as well as fluctuating temperatures. This model is useful because it was specifically validated using a food model (cottage cheese) with competing flora (lactic acid bacteria). It is however only useful for predictions in cottage cheese, not other cheese varieties.

3.1.6 The Raw Milk Cheese Decision Support Tool

The Raw Milk Cheese (RMC) Decision Support Tool is the most comprehensive and appropriate model available to cheesemakers to determine the microbiological safety of their cheesemaking process and finished cheese products. This predictive tool was developed by the Australian Specialist Cheesemakers' Association and Dairy Food Safety Victoria (DFS) through a research project funded by Health Victoria and the New Zealand Ministry for Primary Industries (Quantitative Assessment of Microbiological Safety of Raw milk Cheese Manufacturing) conducted by Kocharunchitt and Ross (Kocharunchitt 2015). Unlike other models based on pathogen growth studies in broth systems, this model was developed using data from challenge studies in actual cheeses, made from both raw and pasteurised milk inoculated with mixtures of *Listeria* spp. and *E. coli* strains (Kocharunchitt 2015).

The tool was developed for cheeses in the following categories, based on the classification scheme of Ottogalli (as per Figure 2) (Almena-Aliste and Miettton, 2014): Hard grating (very hard); hard (Cheddar); semi-hard; internal mold ripened; soft surface ripened; brined; mascarpone; chèvre and cottage/fresh. The developed software allows cheesemakers to use key cheesemaking parameters (pH, A_w , lactic acid and salt) that will support the growth of the sentinel pathogens *S. aureus*, *E. coli* and *L. monocytogenes* in the cheese. These pathogens were deemed to represent the greatest potential risks of growth and survival, in addition to severity of consequences. Since *E. coli* occupies a similar ecological niche as salmonella, and may produce more

severe symptoms associated with illness, *E. coli* was chosen as the surrogate for all pathogenic species among the *Enterobacteriaceae*, including salmonella.

Of note is the fact that the model was designed to determine if raw milk cheese meets the requirements established in Standard 4.2.4 of the Australia New Zealand Food Standards Code¹⁰⁶. Food Standards Australia New Zealand (FSANZ) offers specific guidance for production of raw milk intended for raw milk cheesemaking in Standard 4.2.4. Because of the potential for silage to be a source of *Listeria*, FSANZ does not recommend the feeding of silage to animals in a raw milk herd. If silage is fed, the silage pH must be below 5.0. The Australian Government Proposal P1022 – Primary Production & Processing Requirements for Raw Milk Products specifies production and processing requirements for raw milk used for raw milk cheese manufacture¹⁰⁷.

Using this model, cheesemakers can comprehensively assess the impact of milk quality and handling on cheese safety. Cheesemakers are prompted to input information about their milk quality. Because raw milk quality is essential to insuring the safety of raw milk cheese, if cheesemakers do not enter data on raw milk quality, they will be unable to continue to use the RMC tool. Data cheesemakers must enter on raw milk quality include total plate count at 30°C, and somatic cell counts (expressed as bulk milk cell count (BMCC) in the RMC tool)¹⁰⁸.

Based upon parameters including the temperature of milk two hours following the completion of milking, the temperature of milk during transport, or the milk temperature during storage prior to cheesemaking, along with the duration of storage, the growth potential of *L. monocytogenes*, *E. coli* and *S. aureus* in milk is calculated. In the section dealing with milk tempering, the potential for pathogen growth during milk warming prior to starter addition is calculated. Growth potential during fermentation and moulding is predicted based on time and temperature inputs during these processes. For evaluating the safety of cheese based upon its final characteristics following maturation, the model requests input of pH, A_w , and lactic acid concentration (mM). If cheesemakers do not have these values, they can select the style of cheese they are producing from a drop-down menu on the program. The model makes a predictive assessment of the growth potential of *L. monocytogenes*, *E. coli* and *S. aureus* in cheese. If all three parameters of pH, A_w and lactic acid concentration are available, the model will provide a more accurate assessment of pathogenic growth potential. However, a simpler model can still be used if only pH and A_w data is available.

An overall evaluation is provided that summarises the following parameters for cheesemakers:

- the acceptability of milk quality, handling and transport;
- whether the final product is expected to prevent growth;

¹⁰⁶ <https://www.legislation.gov.au/Details/F2017C00335/Download>

¹⁰⁷ <http://www.foodstandards.gov.au/code/proposals/Documents/P1022-Raw-milk-prods-AppR-SD1.pdf>

¹⁰⁸ <http://www.agriculture.gov.au/SiteCollectionDocuments/aqis/exporting/dairy/goods-for-eu/understanding-eu-requirements-bmcc-tcp.pdf>

- expected levels of growth/inactivation during maturation of *L. monocytogenes*, *E. coli* and *S. aureus*;
- whether the estimated pathogen die-off during maturation exceeds estimated pathogen increases during cheesemaking;
- Whether the process is adequate to ensure production of a safe raw milk cheese.

However, Kocharunchitt and Ross (2015) caution that, with the exception of cheeses that involve a curd cooking step, or Feta style cheese (where safety is achieved by curd cooking to 48°C and an inhibitory pH of 4.4, respectively), the safety of raw milk cheese is primarily dictated by raw milk quality, and not the ability of the cheesemaking process to inactivate pathogens (Kocharunchitt 2015).

3.2 Challenge Testing Methods

Microbiological challenge testing may be used as a means of demonstrating the microbiological safety of a cheese or a process used to make a cheese, but are often used as a last resort when data regarding physicochemical factors preventing growth, microbial ecology and predictive models fail to provide adequate information (Ross 2011).

For data from challenge studies to be valid, the trial must accurately mimic the conditions a challenge pathogen encounters during all stages of cheese production. Many challenge studies conducted on raw milk cheese fail this test by being conducted in pasteurised milk instead of raw milk. Since the majority of raw milk cheeses are produced by small manufacturers, there is likely to be variability in processing from batch to batch (milk quality, pH etc.), and such variability must to be considered in challenge studies so that the range of process efficacy can be determined. The worst case scenario is frequently used to develop conservative interpretations of process efficacy. For the production of realistic results, challenge studies would be ideally performed in actual cheese production facilities. However, due to the dangers associated with introduction of pathogens into processing facilities, surrogate organisms are often used, and they may or may not accurately reflect pathogen behavior (for example, the surrogate must possess the same characteristics as the pathogen, such as acid, salt or heat tolerance, otherwise the results become meaningless), or challenge test studies are performed in laboratories, away from the cheesemaking facility.

Comprehensive guidance for conducting challenge testing for cheese was developed by Ross (Ross 2011) in a technical report entitled “Challenge testing of Microbiological Safety of Raw Milk Cheeses (Challenge Trial Tool Kit)”. Many of the recommendations came from guidelines developed by the U.S. National Advisory Committee for the Microbiological Criteria for Foods (NACMCF 2010) for conducting challenge test studies on pathogen inhibition and inactivation in foods. Ross addresses both considerations as well as some of the limitations and cautions regarding challenge testing. The author cautions that the general applicability of results of challenge studies can be limited and are but only one set of criteria upon which to assess cheese safety. Additional

considerations include the safety record of a particular cheese style, the associated hygienic practices and controls, the degree of reproducibility of the process the knowledge of the associated microbial ecology (which may be inferred from the scientific literature) and predictive models.

The fate of a pathogen is a function of many variables, including:

- the physicochemical properties of the cheese being challenged;
- the associated microbial community and changes in community composition during processing and ripening;
- the conditions of temperature, atmosphere and packaging to which the target pathogen is subjected;
- the environmental limits of growth of the pathogen of concern.

To design a challenge test that will generate reliable and representative results concerning a cheese product or process, there must be an understanding of the pathogen(s) that could contaminate a product, the manner in which contamination could occur, the pathogen load associated with contamination; the physicochemical conditions and microbial communities, along with the duration of processing steps, subsequent handling and post-process exposures, conditions of storage and distribution along with intrinsic properties associated with the cheese in question. Numerous groups have cautioned that the level of contamination, heterogeneity of contamination and physiological state of the bacteria may be difficult to mimic, thus confounding the results of challenge studies. For these reasons, and the associated costs of running a challenge study, it is impractical for most artisan cheese producers to undertake challenge studies. As Ross states “the need for a challenge trial to make a decision about the safety of a product should be carefully evaluated to ensure that the safety of the cheese cannot be resolved in other ways.”

Ross (2011) recommends an initial inoculum of 10^5 cfu/ml of milk based on pathogen levels in raw milk reported in the scientific literature along with practical considerations regarding pathogen fate during processing and the limits of detection associated with enumeration methods. This level exceeds naturally occurring levels of notable pathogens in raw milk intended for artisan cheese manufacture and this may be inappropriate in assessing risk. Furthermore, the microbial ecology of raw milk greatly influences pathogen fate in cheeses made from raw milk. In challenge studies, Kocharunchitt (2015) found that even though Wensleydale and Gouda cheeses belong to the same category of semi-hard cheese, inactivation of *Listeria* differed during ageing of these cheeses, likely due to differences in physicochemical properties. In Gouda cheese, a decrease in *Listeria* populations occurred within the first 40 days of ageing, whereas in Wensleydale, inactivation was not evident within 60 days or more of maturation. Although both cheese styles had a similar pH (5.2-5.5), Gouda cheese had an A_w ranging from 0.920-0.947 versus Wensleydale which had a A_w of 0.961-0.974. These authors also reported, as a result of these challenge studies, that the microbiological safety of a cheesemaking process is based upon parameters that

include: initial pathogen levels, increases in pathogen levels due to growth or concentration due to entrapment in the curd prior to maturation; and inactivation during ageing. From their data, *Listeria* spp. (*monocytogenes* and *innocua*) populations showed a 1-2 log decrease during 60 days of maturation, while *E. coli* populations decreased 2-4 logs, with strain differences observed.

As recommended by Codex (2008) publication of challenge studies conducted by academic and governmental scientists can offer cheesemakers the opportunity to cite appropriate studies as evidence for validation of a cheesemaking process when developing their HACCP plan. Cheesemakers should however be cautious when using these methods as a way of verifying the safety of their own cheeses, as the exact product characteristics and processes may differ from used in the modeling process. They may however provide useful guidance on pathogens might behave in cheese types similar to their own.

3.3 Challenge Testing Results and Considerations

The limitations associated with challenge studies must be acknowledged if they are to be used as evidence supporting the safety of cheese. For instance, most salmonella challenge studies in cheese have been conducted using Cheddar as a model cheese (White and Custer 1976; Leyer and Johnson 1992; Johnson, et al. 1990). Considering that survival of pathogens, including salmonella, in cheese differs with varying manufacturing and ageing parameters and resulting differences in physicochemical characteristics, comparisons should be made with caution. Additional variability in reporting and methodology, among other issues, makes comparisons even more difficult. Studies on the behavior of salmonella in Cheddar highlight the impact of temperature, moisture, pH, acid production and type and amount of starter among other critical factors in the control of pathogens in cheese. These works demonstrated that higher pH and moisture levels, and lower ageing temperatures facilitate comparatively longer survival. When present in large initial populations in milk intended for cheesemaking (5 log cfu/ml), salmonella (*S. Newport*, *S. Newbrunswick*, and *S. Infantis*), survived as long as 9 months in Cheddar cheese (White and Custer 1976).

Similar results were obtained by Park et al. (1970) using lower milk inoculation levels (140-600 cfu/ml) where salmonella survival was observed for 7 and 10 months at 13°C and 7°C, respectively. In this case, pathogen growth and survival from these initial levels was attributed to use of a low acid producing starter culture, producing a cheese with abnormally high pH (5.75 and 5.9) and moisture levels high for a Cheddar (>43%).

With initial counts of 6 log cfu/ml, Hargrove et al. (1969) demonstrated that *Salmonella* spp. survival varied with cheese pH, ranging from 3 months at pH 5 to 6 months at pH 5.3. Similar survival times were reported by Goepfert et al. (1968) for *S. Typhimurium* in stirred curd Cheddar cheese at pH 5.1. Initial counts in raw milk used in cheesemaking (range 1-430 cfu/ml salmonella) increased during manufacture, and then decreased by a factor of 10,000 after 10 to 12 weeks at 13°C, and 14 to 16 weeks at 7.5°C. From

these challenge studies a pH of 5.1 to 5.3 was shown to be sufficient to inactivate salmonella during ageing of semi-hard cheese.

Many challenge studies fail to consider the role of natural raw milk flora that has been shown to have a protective effect against pathogenic bacteria in cheese (Donnelly 2001; Samelis et al. 2009; Montel et al. 2014; Gay and Amgar 2005; Ortenzi et al. 2015). Gay and Amgar (2005) and Ortenzi et al. (2015) compared the fate of *L. monocytogenes* added to raw versus pasteurised milk during the manufacture and ripening of Camembert cheese. The lag phase and time to a 10^3 increase in *L. monocytogenes* levels were twice as long in raw milk Camembert cheese versus the pasteurised counterpart, likely due to the microbiological composition of raw milk, notably thermophilic *Lactobacillus* and yeasts.

In a comprehensive review on cheese rind microbial communities, Irlinger et al. (2015) noted that strains from commercial ripening cultures used for the manufacture of cheeses from pasteurised milk are not necessarily found to be the dominant surface flora in finished cheese. Often “house flora”, (indigenous cultures present in the cheesemaking or cheese ageing environments), rather than added ripening cultures, tend to dominate. This indicates that the microbial composition of cheese is under the strong influence of environmental communities present in the cheesemaking environment (house flora), and ripening cultures often behave differently in complex microbial communities due to their poor adaptation to cheese making processes and their lack of competitive advantage over abundant indigenous flora.

Schvartzman et al. (2011) assessed the fate of *L. monocytogenes* during cheesemaking and ripening of laboratory-made smeared cheeses made with pasteurised or raw cows' milk artificially contaminated with 10^2 cfu/ml *L. monocytogenes*. No growth of *L. monocytogenes* was observed during raw milk cheesemaking, but *L. monocytogenes* increased during the manufacture of pasteurised milk cheeses. In contrast, *L. monocytogenes* grew during ripening of raw milk cheese but was inactivated in pasteurised milk cheese. The authors attribute this finding to higher levels of background microflora in raw milk that limited starter culture LAB activity, resulting in higher cheese pH values. The study confirmed the importance of challenge testing for improving specific knowledge of pathogen fate (growth, inactivation or stagnation) during cheesemaking and ripening, because beyond pH, specific microbial communities may have protective, antagonistic or lethal effects beyond the metabolic products that they produce.

D'Amico et al. (2008) compared the survival of *L. monocytogenes* on surface mould-ripened soft cheeses manufactured from raw or pasteurised milk and held for ≥ 60 days at 4°C. Final cheeses met the U.S. Federal Standards of Identity (21 CFR Part 133)¹⁰⁹ for soft ripened cheese, with low moisture targets to facilitate the holding period. After brining and drying, cheese wheels were surface inoculated with a 5 strain cocktail of *L.*

¹⁰⁹ <https://www.gpo.gov/fdsys/granule/CFR-2012-title21-vol2/CFR-2012-title21-vol2-part133/content-detail.html>

monocytogenes to contain 0.2 cfu/g (low level) or 2 cfu/g (high level) and ripened for 12 days at 14°C +/- 1°C at 90% +/- 2% relative humidity (RH) to allow surface flora growth. Wheels were then wrapped and held at 4°C +/- 1°C for 57 days. Weekly, duplicate 1cm deep surface samples (100g) were removed, diluted, surface plated on CHROMagar *Listeria* selective agar, incubated and enumerated. After an initial decline to undetectable levels, growth commenced at day 28 for both contamination levels and reached 3 log cfu/g and 5 log cfu/g after 70 days for low and high level inoculations, respectively. No significant differences ($p < 0.05$) were observed in pH development, growth rate, or population levels between the milk types. Lower moisture soft ripened cheeses held for 60 days supported the growth of very low initial levels of *L. monocytogenes* when introduced as a post-process contaminant independent of the milk type used for manufacture.

The safety of cheeses within this category must therefore be achieved through alternate control strategies that include limiting refrigerated cheese storage time i.e. shortening shelf life, prevention of post-process recontamination through use of optimal sanitation, use of raw milk meeting stringent microbiological criteria or use of pasteurised milk in cheesemaking. It is notable that France requires the ageing of Camembert de Normandie cheese for a minimum of 21 days, but restricts the sale of this cheese beyond 55 days (Sanaa et al. 2004). The U.S. FDA has recently requested scientific data and information to assist the agency in identifying and evaluating intervention measures that might have an effect on the presence of bacterial pathogens in cheeses manufactured from unpasteurised milk. The FDA is taking this action in light of scientific data on potential health risks associated with consumption of cheese made from unpasteurised milk.

Hammer et al. (2017) recently examined the fate of *L. innocua* during production and ripening of hard smeared rind cheese (Gruyère) manufactured from raw milk. *L. innocua* has been shown to be a suitable surrogate for *L. monocytogenes* (Brita 2017) and was chosen as a surrogate for pathogenic strains of *L. monocytogenes*, with initial populations added at levels of 10^5 cfu/g. Curd cooking for 2 hrs at 56°C reduced populations below 10^2 cfu/g after 24h, and holding at 50°C for 70 min resulted in further reductions of *Listeria* populations. Counts in cheese cores were reduced to 10^3 cfu/g within 12 weeks of ripening and *Listeria* was undetectable after 24 weeks of storage. Within the rind, however, high populations of *Listeria* (10^6 - 10^8 cfu/g) were detected, and these levels remained stable throughout ripening. The smear culture, comprised of the bacterial species *S. equorum*, *Corynebacterium casei*, *Brevibacterium linens* and *Microbacterium gubbenense*, and yeasts *Debaryomyces hansenii* and *Geotrichum candidum*, displayed no anti-listerial activity. The curd pH at cutting was 6.56-6.58, and this was reduced to pH 5.11-5.19 following pressing. During ripening, cheese pH ranged from 5.34-5.57 and was 5.74-5.93 in the cheese core at the end of ripening. The study authors noted that contaminated rinds could introduce *Listeria* to the cheese core during cutting, emphasising the importance of control of surface contamination by *Listeria*. Rinds are subject to the same microbiological criteria as the core – with a few exceptions (e.g. the rind of Gorgonzola is stated as being inedible in the PDO

specification as it is considered to be the primary packaging). However, it must be remembered that even though they would not typically be consumed, when a rind is contaminated with *Listeria*, and a knife is drawn through the rind into the core to cut the cheese, the cheese core can become contaminated.

D'Amico et al. (2010) compared the fate of *E. coli* O157:H7 during the manufacture and ageing of Gouda and stirred-curd Cheddar cheeses made from raw milk. Cheddar and Gouda cheeses were manufactured from raw milk experimentally contaminated with *E. coli* O157:H7 to simulate the scenario of milk contamination followed by bulk tank refrigeration before cheesemaking. Cheeses were manufactured in a lab scale cheese vat from unpasteurised milk that was inoculated with one of three strains of *E. coli* O157:H7 at an approximate concentration of 20 cfu/ml. Samples of milk, whey and curd were collected for enumeration throughout cheesemaking process. Finished cheeses were vacuum packaged and aged at 9°C ± 1°C. Cheese samples were removed for detection and enumerated at set intervals during the ageing period until *E. coli* O157:H7 was no longer detected by selective enrichment. Overall, counts in both cheese types increased almost 10-fold from initial milk inoculation levels to an approximate concentration of 145 cfu/g in cheeses on day one. From this point, counts dropped significantly over 60 days to mean concentrations of 25 and 5 cfu/g of Cheddar and Gouda, respectively. Levels of *E. coli* O157:H7 fell and stayed below the cultural detection limit of ≥5 cfu/g after an average of 94 and 108 days in Gouda and Cheddar, respectively, yet remained detectable following selective enrichment for more than 270 days in both cheese types. Changes in pathogen levels observed throughout manufacture and ageing did not significantly differ by cheese type. In agreement with previous studies, results suggest that a 60-day ageing requirement (see Chapter 1) alone is insufficient to completely eliminate viable levels of *E. coli* O157:H7 in Gouda or stirred-curd Cheddar cheese when manufactured from raw milk contaminated even with low initial levels of this pathogen.

In a challenge test study, Kasrazadeh and Genigeorgis (1995) showed that soft Hispanic type cheese having a pH value of 6.6, moisture of 60%, low brine of 1.61% and manufactured without the use of starter culture is an excellent substrate for the growth of enterohaemorrhagic *E. coli* if the storage temperature exceeds 10°C or more. No growth of *E. coli* O157:H7 occurred during a two-month period when cheese was stored at 8°C.

The behaviour of *Escherichia coli* O157:H7 was studied during the manufacture and ripening of a soft smear-ripened Irish farmhouse cheese produced from raw milk (Maher et al. 2001). The results indicate that the manufacturing procedure encouraged substantial growth of *E. coli* O157:H7 to levels that permitted survival during ripening and extended storage. While declines in population levels during ripening were noted, surviving populations were still found to exist in the cheese core after 6 weeks, the normal expiration date of this product.

Survival and growth characteristics of *Escherichia coli* O157:H7 in pasteurised versus unpasteurised Cheddar cheese whey at two initial inoculation levels (10² and 10⁵ cfu/g) was explored by Marek et al. (2004). Survival of *E. coli* O157:H7 was found to be

significantly higher in pasteurised whey samples at all storage temperatures (4, 10 or 15°C) compared with unpasteurised whey samples. Lactic acid bacteria in unpasteurised whey had an inhibitory effect on *E. coli* O157:H7. Initial populations of lactic acid bacteria in unpasteurised whey samples (approximately 10^7 cfu/ml) survived, and at day 28, greater than 10^3 cfu/ml of lactic acid bacteria were present in unpasteurised whey at all temperatures, with the highest counts recovered at 4°C. Significant growth of *E. coli* O157:H7 was seen in pasteurised whey stored at 10 and 15°C, with no detectable LAB in these samples. The results indicate the potential risk of persistence of *E. coli* O157:H7 in pasteurised whey in the event of contamination with this pathogen post-pasteurisation, e.g. through cross contamination, highlighting the need for stringent sanitary practices during whey storage and handling.

Survival of *Salmonella* Typhimurium and *Escherichia coli* O157:H7 was studied in model brines used for salting alongside brine from three cheese plants by Ingham et al. (2000). Results of this study show that cheese brine could support the survival of contaminating *S. Typhimurium* and *E. coli* O157:H7 for several weeks under typical brining conditions (consisting of 23% NaCl with or without added whey) emphasising the need for strict hygiene of the brine as discussed in section 2.2.5.3.

Montet et al. (2009) compared the ability of acid resistant and non-acid resistant (NAR) STEC strains to survive during Camembert cheese manufacture. Numbers of STEC increased from 1-2 logs at the beginning of cheese manufacture, most likely due to entrapment in the curd and concentration during whey drainage. Populations were found to reach a plateau at the end of cheese moulding, where the pH reached 4.65, until the end of the drying stage. Thereafter, from the middle of ripening (10 days), where cheese reached a pH of 4.75 to the end of ripening at 20 days, where the pH reached 5.11, STEC levels showed an approximate 1.5-3.0 log decrease. Montet et al. (2009) did not find induction of acid adaptation of the four NAR STEC strains by the acidic conditions encountered during lactic cheese production (4.65-4.75), and subsequent exposure to simulated gastric fluid resulted in rapid destruction of STEC. Initial levels of STEC (10^3 cfu/ml) were reduced to 10 cfu/g at 20 days of ripening (2 log decrease). As the authors have shown, both acid-resistant and non-acid resistant STEC can potentially survive artisanal Camembert cheese manufacture; therefore good milk hygiene is essential for the manufacture of Camembert.

A study conducted by Reitsma and Henning (1996) examined survival of *E. coli* O157:H7 during the production and ageing of Cheddar cheese. In this study, the authors inoculated pasteurised milk with *E. coli* O157:H7 at two levels: 1000 cfu/ml (treatment 1) and 1 cfu/ml (treatment 2), and followed the pathogen in cheese over 158 days of ageing. A 2 log reduction of *E. coli* populations was recorded after 60 days of ripening in treatment 1, though viable cells were still recovered at 158 days. In contrast, no viable cells were recovered after 60 days of ripening, even though *E. coli* O157:H7 was present at levels of 60 cfu/ml in curd after salting in treatment 2. However, the experimental design used by these study authors has a number of flaws. They erroneously used pasteurised milk, a problem encountered in many challenge studies

that attempt to study the fate of pathogens in cheeses manufactured from raw milk. Additionally, the cheese manufactured in this study had salt in the moisture phase (SMP) levels that ranged from 2.75-3.76%, with a mean 3.25%, compared to normal Cheddar where the average SMP is 5-5.5%. The low SMP and absence of natural inhibitors in raw milk could have created an artificially protective environment for *E. coli* O157:H7. Despite this, *E. coli* O157:H7 present at 60 cfu/g in curd after salting was reduced to <1 cfu/g during 60 days of ageing showing that Cheddar cheese has an environment that facilitates reduction of *E. coli* O157:H7 populations provided that initial levels in cheese are low.

In a follow-on study, Schlessner et al. (2006) examined the fate of *E. coli* O157:H7 in Cheddar cheese made from unpasteurised milk inoculated with 10^1 to 10^5 cfu/ml of a five-strain cocktail of acid-tolerant *Escherichia coli* O157:H7. Samples collected during the cheese manufacturing process showed increases in populations of *E. coli* O157:H7 during cheesemaking. *E. coli* O157:H7 populations in cheese aged for 60 and 120 days at 7°C decreased less than 1 and 2 logs, respectively. While these studies confirm previous reports that show 60-day ageing is inadequate to eliminate *E. coli* O157:H7 during cheese ripening, there are concerns used in the experimental design of these challenge studies. The authors added *E. coli* O157:H7 at extremely high population levels (up to 10^5 cfu/ml) to milk used for cheesemaking. At the time of this study, the U.S. FDA set standards for EHEC (10^3) and *E. coli* (10^4) in cheese (Guide 7106.08¹¹⁰) and the manufactured cheese did not conform to these standards, which have been subsequently revised (Donnelly 2001). FDA issued updated instructions to its field laboratories to limit testing for non-toxigenic *E. coli* in raw milk cheese to solely five (5) sub-samples and regulatory information that lots exceeding 10 MPN/g and less than 100 MPN/g in three or more sub-samples of the five examined are not acceptable; not two or more subsamples as presented in 2010 CPG. Changes to FDA's Compliance Program Guidance for Domestic and Imported Cheese Products are underway to reflect this adjustment. However, as of this writing, FDA has announced that it "is in the process of pausing" its testing program for generic *Escherichia coli* ("*E. coli*") in cheese based largely on the fact that this testing did not result in any public health benefit. The results highlight the importance of adherence to raw milk cheese microbiological standards.

Minas cheese is a Brazilian fresh cheese that can support the growth of bacterial pathogens due to its high moisture content, low salt content and pH that ranges between 5.0-7.0. Control of pathogens in this cheese is based solely on use of refrigeration and restrictions on shelf life (14 days). The addition of lactic acid bacteria (LAB) for pathogen control is an optional practice. Eight different formulations of Minas cheese were manufactured using raw or pasteurised milk and with or without salt and LAB cultures (Saad et al. 2001). Individual portions of each formulation were transferred to sterile plastic bags and inoculated with *E. coli* O157:H7 at initial levels of 10^3 or 10^6 cfu/g. *E. coli* O157:H7 counts in samples without added LAB cultures showed a 2-log increase in the first 24 h and remained constant during 14 days of storage. In contrast,

¹¹⁰ Guide no longer available on FDA website

counts in samples with added LAB culture showed a 0.5-log increase during the first 24h, followed by a decrease, and results were statistically significant ($p < 0.05$). No significant variations were found for cheeses manufactured with pasteurised or raw milk, with or without salt. Results indicate that the addition of a mesophilic homofermentative type O LAB commercial culture (*Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*) may provide additional safeguards to GMP and HACCP programs in controlling *E. coli* O157:H7 in Minas cheese.

Miszczucha et al. (2013) examined behavior of eight different strains representing four different STEC serotypes (O157:H7, O26:H11, O103:H2, and O145:H28) at target inoculation levels of 10^2 cfu/ml in milk before rennet addition during the manufacture of five cheese types. The five cheese types consisted of a blue-veined cheese made from raw ewes' milk (blue type cheese; BTC); a lactic cheese made from raw goats' milk (LC); a cooked cheese made from raw cows' milk (CC); and two uncooked pressed cheeses made from raw cow's milk (UPC1 and UPC2). During BTC manufacture, STEC O157:H7 increased by 1 log during the first 24h, whereas O26:H11 and O103:H2 strains increased by 3 logs and 2 logs, respectively. Seven days after coagulation, O157:H7 strains decreased rapidly and were only detected by enrichment. By 240 days no detection of O157:H7 was evident even when enrichment was used. In contrast, levels of O26:H11 decreased rapidly and were only detected through enrichment at 240 days but were found to grow more rapidly and be more persistent than O157:H7 and O103:H2 strains. O101:H2 decreased during ripening and could not be detected at 240 days. Physicochemical analysis showed a decrease in the pH of the cheese core from 6.6 to 4.91 during the first 3 days, then an increase until day 90 when it reached pH 6.69, then it decreased again below pH 6.0, reaching approximately pH 5.5 at day 240. The A_w decreased to 0.898 at day 240. In LC, O103:H2, O145:H28 and O157:H7 populations remained consistent with initial levels in raw milk. O26:H11 strains increased by 1 log, then decreased up to the end of coagulation at 24 hours. The increase in O26:H11 was significantly higher than other serotypes examined. At end of moulding (60h), all strains were detectable only by enrichment. STEC remained detectable during ripening and storage, but at day 60, four strains (O26:H11, O103:H2, and two O157:H7) were not isolated even with enrichment; only O145:H28 remained detectable.

In the same study, physicochemical analysis showed a rapid decrease to pH 4.21 at day 2, followed by an increase to pH 5.26 at day 25. On day 45, the A_w had decreased from 0.994 to 0.967. For CC, no growth of STEC serotypes was observed during the first hours of cheesemaking, and throughout ripening, strains were only observed after enrichment. At day 120, only one of two O157:H7 strains could be isolated after enrichment from core samples. Physicochemical analysis showed a pH decrease from pH 6.6 to pH 5.38 in the cheese core, where it remained stable for 1 month, then pH slowly increased to reach pH 5.82 at day 120. Water activity (A_w) readings were stable between days 5-15, then decreased to reach 0.975 in the core at day 120. The rind A_w decreased to 0.964 at day 120. With UPC1 with a long ripening step (LR), *E. coli*

O26:H11 increased to 6 logs during the first 24 hours of cheesemaking; O157:H7 strains reached 4 logs. The STEC population remained constant between day 1 and day 60. Levels declined during ripening and after 210 days dropped below enumeration limits for O157 in the rind and core, and in the rind for O26:H11; levels decreased more slowly in the core versus the rind and were still present at 3 logs at day 240. Physicochemical data showed the cheese core pH decreasing from pH 6.79 to 5.19 during the first 24 hours, then pH increased to 5.52 at day 240. The A_w decreased in core to reach 0.943 at day 240, 0.941 at day 60, and to 0.922 at day 240 in rind. In UPC2-with a short ripening step (SR) all four STEC serotypes increased during the first 24h of manufacture. O103:H2 and O145:H8 reached 5 logs. O26:H11 reached 4-5 logs; O157 3.3 logs. Populations remained constant until day 40; no differences existed in the rind and the core. Traditional ripening is done for 20 days compared with industrial ripening that is done for 12 days.

Physicochemical analysis showed the cheese core pH decreased from 6.6 to 5.3 on the first day, then increased slowly after day 5 to reach 5.80 at day 40. The A_w in the core remained at 0.974 at day 40 while the rind A_w was 0.980 at day 40. The extended ripening was conducted to verify the results of a previous study conducted by Vernozy-Rozand et al. (2005). For all cheese types examined, two factors were found to inhibit growth of STEC in first hours of cheesemaking: sudden rapid acidification (LC-decrease to pH 4.3) and high temperature (CC-54°C for 35 min). Vat pasteurisation is 62.8°C for 30 min (equivalence to pasteurisation would be organism dependent, but 54°C for 35 min would deliver substantial kill of vegetative bacterial pathogens). The minimum A_w needed for STEC growth is 0.95 (Lindblad and Lindqvist 2010). Negative temperatures combined with decrease of A_w and acidic pH may reduce populations, as observed with UPC1-LR. Short ripening periods (UPC2-SR) could not achieve a significant A_w reduction. Miszczycha et al. (2013) concluded that the survival of STEC in raw milk products may be affected by a combination of factors (time, pH, A_w and temperature). STEC strains O26:H11, O103:H2 and O145:H28 may be better competitors than O157:H7¹¹¹. The results from this study demonstrate that a heating step and sudden rapid acidification allow efficient STEC removal for CC and LC. Moderate acidification and moderate temperatures allow growth in BTC and two UPCs, but long ripening can reduce levels. Duffy, et al. (1999) and Miszczycha et al. (2013) suggested autochthonous milk microflora and starter LAB and a range of moulds play an antagonistic role against STEC.

In the Draft Assessment Report for an application to permit the sale of Roquefort Cheese in Australia¹¹². FSANZ concluded that the cheesemaking process and subsequent maturation achieved a significant reduction of STEC. In this report, challenge studies conducted by the Pasteur Institute are summarised. Populations of *E.*

¹¹¹ N.B. *E. coli* O157 can be used as a proxy for all STECs as there is not yet conclusive scientific evidence regarding how different STECs may behave in different cheese types. The important factor is to insure a control step that assures conditions that would not permit survival of any STEC.

¹¹² http://www.foodstandards.gov.au/code/applications/Documents/A499_Roquefort_FAR_FINALv2.doc

coli O157:H7 reaching levels of >3,000 cfu/g declined following salting and were not detected through enrichment beyond ninety days of maturation. The report states that “Confirmation that the final product achieves a moisture content of 43-45% (often reported as 55-57% dry matter) and a salt concentration of 3.6-4.3% provides similar assurance regarding availability of moisture in the final product” and “An extended ripening/maturation period for Roquefort cheese was identified as an important processing measure contributing to the safety of this product. A minimum storage time of 90 days has been recommended.” Therefore, salting and ageing should be considered critical control points (CCP) in Roquefort cheese production.

The fate of *Escherichia coli* O157:H7 was investigated during the manufacture of Mozzarella cheese (Spano et al. 2003). Mozzarella cheese was made from unpasteurised milk which was inoculated to contain approximately 10⁵ cfu/ml *E. coli* O157:H7. The results show that stretching curd at 80°C for 5 min (milk pasteurisation is 71.7°C for 15 sec) is effective in controlling *E. coli* O157:H7 during the production of Mozzarella cheese. Brining and storage at 4°C for 12 h was found to be less effective than the stretching. Mozzarella cheese should be free of *E. coli* O157:H7 if time and temperature parameters of 80°C for 5 minutes are used during curd stretching. Similar findings were obtained by Buazzi et al. (1992) who found that *L. monocytogenes* failed to survive during the manufacture of Mozzarella cheese.

Voysey et al. (2012) assessed the fate of VTEC in Caerphilly cheese. The experimental Caerphilly cheeses made by these authors were broadly within typical physicochemical ranges with the exception of moisture content, which ranged from 47.9-53.6% instead of the literature range of 33.9-46.0%. As the authors note; “Because of the high moisture levels in the cheeses, the findings that there was an increase in VTEC concentration during cheese manufacture and there was a slow decline in count over the life of the Caerphilly cheese, have to be questioned.”

3.4 Summary Findings from Chapter 2

A number of predictive models are available to assist cheesemakers in safety assessments by predicting their growth potential in cheese, with the recently developed Raw Milk Cheese Decision Support Tool being the most appropriate and user-friendly model for artisan cheesemakers. Predictive models may over-predict pathogen growth and survival. These tools, however, can serve as important guidance in a first step towards assessment of risk of potential for growth of pathogens in cheeses made from unpasteurised milk, and identification of growth potential of pathogens for shelf life predictions to enhance safety.

Challenge studies have shown that the microbiological risk of cheeses made from unpasteurised milk can be greatly reduced by a proper acidification, ripening (maturation) process and constant monitoring of the hygiene environments for milk production, cheesemaking and the post-manufacturing stage. Scientifically documented time and ripening temperature combinations and acidification processes can prevent the

growth of unwanted and potentially harmful bacteria that may cause spoilage and foodborne disease. While some challenge studies have shown that 60-day ageing can be conducted for improving the microbiological quality of some raw milk cheeses made from unpasteurised milk, other studies have demonstrated that 60-day ageing may not be effective against existing *E. coli* O157 and therefore a risk of foodborne illness may still exist. Other challenge studies document that foodborne pathogens including *L. monocytogenes*, *Salmonella* spp. and *Staphylococcus aureus* can be inhibited by the naturally occurring bacteriocinogenic lactic acid bacteria (LAB) found in unpasteurised milk. The interactions between physicochemical conditions (such as pH or A_w) and natural microflora in the production of cheeses made from unpasteurised milk, and how the resulting conditions impact on the survival and growth of pathogens is not fully understood.

As current microbiological modelling programmes do not typically take into account the competition between pathogens and unpasteurised milk microflora, it is challenging for cheese producers, in the absence of challenge studies, to demonstrate how pathogens are being controlled through their production process. Given the cost and necessary scientific expertise required, cheesemakers are unlikely to routinely conduct challenge studies and this information must therefore be obtained through academic research, technical consultants and guidance from food safety authorities.

4.0 CHAPTER 3: An analysis of historical microbiological and physicochemical results obtained from cheesemakers undertaking sampling in their products

4.1 Summary of recommended limits of microorganisms for milk and cheese

Cheesemakers are required to conform to legal requirements to assure the safe production of cheese. The legal requirements for UK cheesemakers with regards to the microbiological safety of raw milk and for cheese are listed in Tables 36, 37, 38 and 39, respectively. Adherence to legal standards alone, however, will not always assure safe cheese production. To that end, the SCA ACoP¹¹³ (2015) offers guidance on microbiological targets or levels that producers should aim for during milk production and cheesemaking. The SCA note that adherence to the recommended target levels allows day to day variation in microbiological counts without exceeding regulatory criteria.

It is important that testing be done as frequently as needed to assure adequate process control -as determined by the cheesemaker as part of their food safety management system. For cheeses that do not support the growth of pathogens due to their physicochemical characteristics, testing is likely to be required less frequently than for high moisture, high pH cheeses that are more likely to pose food safety risks. Additionally, as noted by D'Amico *"regular bacteriological analyses of both milk and cheese play an integral role in the control and prevention of pathogens and subsequent outbreaks because the physicochemical parameters of some cheeses permit the survival and possible growth of certain pathogens."* The SCA (2015) provide information on factors to consider when designing a sampling regime.

It is also recommended that physicochemical compositional data, particularly pH and salt-in-moisture values be analysed in a similar way. Microbiological and physicochemical data taken together can be useful to verify that a HACCP plan is operating as intended. The SCA notes that given the complexity of cheesemaking and the diversity of processes employed, specific recommendations can only be made on a case-by-case basis.

4.2 Analysis of historical microbiological and physicochemical results obtained from cheesemakers

4.2.1 Raw milk microbiological data from the Specialist Cheesemakers Association

The SCA conducted a survey of the microbiological quality of raw milk used in cheesemaking by UK SCA members. A total of 1,076 samples representing 31 cheese producers were obtained between January 2011 and August 2012. Participating cheese

¹¹³ <http://www.specialistcheesemakers.co.uk/assured-guide.aspx>

producers were spread geographically around the UK, with 23 representing England, 4 from Wales and 4 from Scotland. Milk came from diverse animal species and breeds including Friesian and Jersey cows, sheep and goats, and farms represented both conventional and organic production practices. Nineteen different laboratories performed analyses for salmonella, *Listeria*, coagulase positive staphylococci, *E. coli* (generic), STEC O157:H7, coliforms and Enterobacteriaceae. Analyses performed were risk based and not all samples were tested for all organisms. Table 36 depicts results of milk analysis for pathogens. In agreement with previous surveys (D'Amico et al. 2008) the incidence of pathogens was low, with absence of *Salmonella* and *E. coli* O157:H7 in 298 and 225 tested samples respectively.

Table 36. SCA raw milk testing results 2011-2012: analysis of pathogens of concern (*L. monocytogenes*, *Salmonella enterica* and STEC O157)

Organism	No. Tests	Detected/ 25ml		<20/ ml		Highest Count (cfu/ml)
		No. samples	%	No. samples	%	
<i>L. monocytogenes</i>	639	43	6.7	14/14	100.0	<20
<i>Listeria</i> spp. (other than Lm)	639	40	6.3	n/a	n/a	n/a
<i>Salmonella enterica</i>	298	0	0.0 (<0.3%)**	n/a	n/a	n/a
<i>E. coli</i> O157	225	0	0.0 (<0.4%)	n/a	n/a	n/a

* n/a = not available

** Numbers in brackets depict those values which were below detection limits

L. monocytogenes was detected in 6.7% (43/639) of analysed samples. This is a high incidence of *L. monocytogenes* in milk in comparison with other surveys that have examined the microbiological quality of milk specifically used for artisan cheese production. The SCA noted that during the survey, two cheesemakers experienced incidents involving *L. monocytogenes*, which elevated the level of testing and concomitant numbers of isolates that contributed to the high prevalence reported. D'Amico and Donnelly reported *Listeria* prevalence rates of 4.8% for cows' milk and 2.3% for cows', goats' and sheep milks combined in a 2006 study (D'Amico et al. 2008) and 0% (0/101 samples) in a 2008 study (D'Amico and Donnelly 2010). These findings highlight the need for focus on sources of *Listeria* contamination, as once identified, these sources can be effectively eliminated. Given the well-documented association between silage feeding and shedding of *L. monocytogenes* into milk (Arimi et al. 1997; Ryser et al. 1997), feeding regimes used on farms producing milk for artisan production in the UK warrant investigation.

In addition to these pathogens, milk samples were analysed for coagulase-positive staphylococci, generic *E. coli*, coliforms and *Enterobacteriaceae*. Table 37 depicts results of SCA's 2012 analysis of raw cows' milk. The majority of tested milk samples

(91%) had coagulase-positive staphylococci at levels below detection limits (<20/ml). The prevalence rate of 9% (47/548) for coagulase positive staphylococci is lower than the prevalence rates of 35% (46/133) and 38% (38/101) obtained by D'Amico and colleagues in surveys of U.S. raw milk (D'Amico et al. 2008; D'Amico and Donnelly 2010). This could be due to the fact that these authors were specifically enumerating *S. aureus* in a 1 ml test aliquot versus the SCA method that examines 0.5 ml of a 1:10 dilution of milk sample, with the former method providing a higher degree of sensitivity (although the results are comparable). Despite this, the low levels of coagulase positive staphylococci obtained by the SCA (<20/ml) in the majority of tested samples agree with results of D'Amico (D'Amico et al. 2008; D'Amico and Donnelly 2010) who found a mean levels of *S. aureus* of 25 cfu/ml in 2008 surveys and 20 cfu/ml in 2010 surveys. Similarly, 16% of 551 cows' milk samples had detectable levels (>20 cfu/ml) of generic *E. coli*, with 2% of samples having levels exceeding 1000 cfu/ml. The majority (84%) of samples (462/551) had undetectable *E. coli* (levels of <20 cfu/ml), reaching lower limits than those defined in the regulations and showing adherence to good hygienic practices.

Table 37. Results from SCA survey of microbiological quality of raw milk (Jan. 2011-Aug. 2012. (SCA Technical Committee, October 2012).

Organism	No. Tests	<20 cfu/ml		20–100 cfu/ml		100-1000 cfu/ml		>1000 cfu/ml		Highest Count (cfu/ml)
		No. samples	%	No. samples	%	No. samples	%	No. samples	%	
Coagulase-positive staphylococci	548	501	91%	21	4%	24	5%	2	0%	1,120
<i>E. coli</i> (generic)	551	462	84%	56	10%	21	4%	12	2%	6,000
Coliforms	403	232	58%	101	25%	46	11%	24	6%	100,000
Enterobacteriaceae	236	105	45%	76	32%	43	18%	12	5%	100,000

The SCA recommends that cheesemakers aim for <100 cfu/ml of coagulase-positive staphylococci in raw milk. Of 548 samples tested for coagulase positive staphylococci, 26/548 samples (4.7%) exceeded these criteria (>100 cfu/ml), showing an area where improvements in milk safety could be made.

Similarly, the SCA recommends that cheesemakers aim to have <100 cfu/ml of generic *E. coli* in raw milk (SCA, 2015 (table 5.2.1)). A total of 33/551 samples (6.0 %) exceeded these criteria, again highlighting an area where improvements in milk hygiene

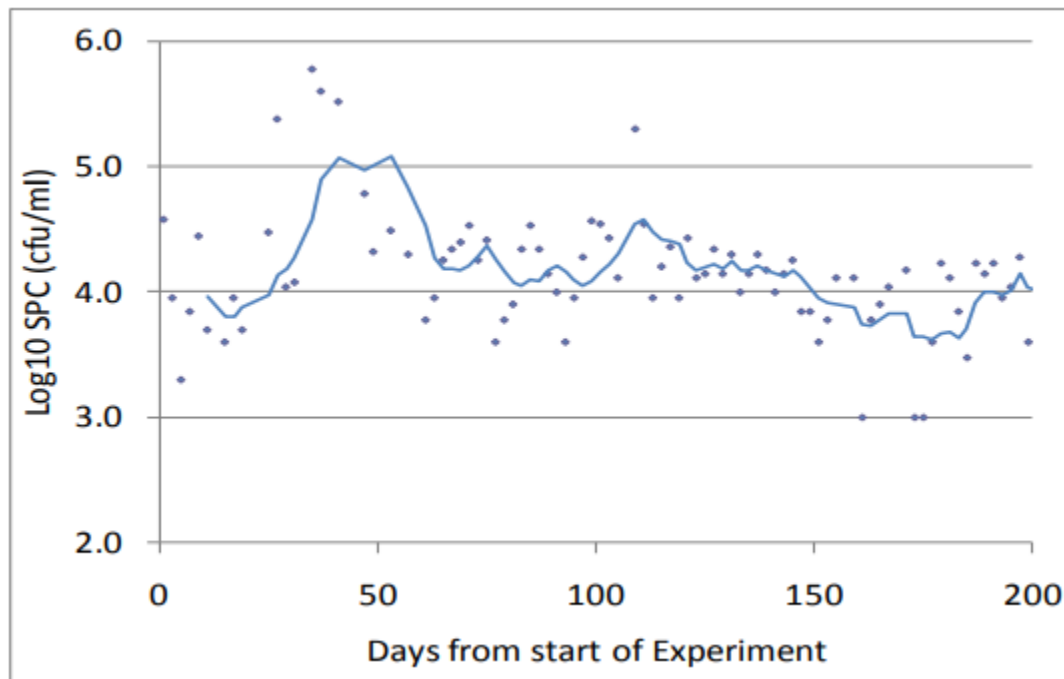
can be made. The SCA recommends that cheesemakers aim for <100/ml coliforms in raw milk. 70/403 samples (17.4%) exceeded SCA targets, again indicating that improvements in hygiene and sanitation may be needed to achieve these targets. Enterobacteriaceae (EB) at levels of >10,000 cfu/g in cheese are unsatisfactory according to UK HPA Guidelines (Nov 2009). In order to achieve this target, levels in milk should be kept at <1000 cfu/ml. During cheesemaking, whey removal concentrates levels of organisms in the curd due to entrapment, increasing levels approximately 10-fold. The SCA data indicates that 5.0% of milk samples analysed would not yield cheese below the 10,000 cfu/g EB target.

4.2.2 Microbiological trend analysis

There are few published studies in the scientific literature that specifically address historical microbiological results obtained from cheesemakers. As a result, it is difficult to note trends or spikes, and as indicated by the SCA (2015), this is normally done on a case by case basis. There are reports, however, that offer advice on preferred methods for cheesemakers to analyse trends in their routine sample results. Reinemann (2011)¹¹⁴ recommended use of a moving average trend line as a way to visualise trends in counts over time. In the data shown in Figure 12 below, daily counts (new batch every day) of log₁₀ SPC cfu/ml are depicted as symbols (◇) as a time series plot. Reinemann (2011) recommends log transformation of bacterial counts to convert numbers to a more normally distributed population that will provide a better estimate of increased bacteria counts on milk quality effects. Log transformation also provides a more accurate assessment of deviations over time and should always be used when averaging or exploring data trends. Unfortunately, trends are not easily observable in this format. In contrast, the addition of a 5 day moving average trend line in the Excel graph easily allows visual analysis (Figure 12). The one cautionary note is that viewing trend analysis alone may mask the highest spikes, as shown for 5 samples where standard plate counts exceed 5.0 log₁₀ cfu/ml. In this case, immediate corrective action is needed to bring levels back to SCA recommended limits (4.0 log₁₀ cfu/ml).

¹¹⁴ https://milkquality.wisc.edu/wp-content/uploads/sites/212/2011/09/troubleshooting_machine-or-cows.pdf

Figure 12: Moving average trend line analysis of SPC counts in raw milk



*SPC = Standard Plate Count

4.2.3 Dairy Food Safety Victoria Guidance

Dairy Food Safety Victoria (DFSV) issued guidance¹¹⁵ in 2015 for minimum recommended test requirements for manufacturers of dairy foods based on historical trends and the risk profile of different dairy products. Target organisms and limits are described below in Table 38. This guidance states that while it is desirable to test every product lot and batch against the relevant microbiological criteria recommended in the guide, DFSV recognises the significant burden this can place on small scale dairy producers, therefore, the guidance offered suggests a minimum testing frequency of every 10 batches for soft and semi-soft cheese, which are considered high risk given that they contain >39% moisture. For all other cheeses having <39% moisture, testing is recommended every 20 batches. DFSV also recognise that in cases where products are manufactured infrequently, an extended period of time may occur before every 10 or 20 batches are tested, and in this case, testing should occur at least once every two months. DFSV state that tracking microbiological test data allows manufacturers to demonstrate process control and also identify emerging issues or trends which may result in the need for additional testing.

¹¹⁵ <https://www.dairysafe.vic.gov.au/resources/guidelines/347-dfsv-micro-testing-criteria/file>

Table 38: Microbiological testing criteria for cheese as recommended by Dairy Food Safety Victoria 2016

Cheese type	Microorganism	Limits	DFSV Minimum Requirements	
			Sampling	Frequency
Soft and semi soft cheese >39% moisture, pH >5.0	Coagulase-positive staphylococci cfu/g	n=5 c=2 m=100 M=1000	1 sample (Limit: 100 cfu/g)	Every 10 batches
	<i>E. coli</i> cfu/g	n=5 c=1 m=10 M=100	1 sample (Limit: 10 cfu/g)	Every 10 batches
	Salmonella/25g	n=5 c=0 Not detected/25g	5 samples composited (Limit: ND/125g)	Every 10 batches
	<i>Listeria monocytogenes</i> /25g	n=5 c=0 Not detected/25g	5 samples composited (Limit: ND/125g)	Every 10 batches
All cheese (<39% moisture)	Coagulase-positive staphylococci cfu/g	n=5 c=2 m=100 M=1000	1 sample (Limit: 100 cfu/g)	Every 20 batches
	<i>E. coli</i> cfu/g	n=5 c=1 m=10 M=100	1 sample (Limit: 10 cfu/g)	Every 20 batches
	<i>Listeria monocytogenes</i> /25g	Based on product supporting/does not support growth	Recommended 5 samples composited and tested	Every 20 batches

4.2.4 Pro forma for Raw Milk Cheese Processes: New South Wales (NSW) Department of Primary Industries

In 2012 changes were made to the Food Standards Australia New Zealand (FSANZ) Dairy Standard ¹¹⁶to enable the production and sale of hard to very hard, cooked curd cheeses made from raw milk. In February 2015, these changes became law. A number of tools have been developed to help manufacturers of raw milk cheese comply with the new regulations. For instance, in order to manufacture cheese in New South Wales (NSW) Australia, a business must be licensed and the raw milk cheese manufacturing

¹¹⁶ <http://www.foodstandards.gov.au/code/primaryproduction/dairy/Pages/default.aspx>

process must be approved by the NSW Food Authority. Raw milk cheesemakers must complete a production process proforma¹¹⁷ to demonstrate that they can source high quality raw milk that complies with milk sanitary standards, that cheese placed on the market will not support the growth of pathogenic bacteria (particularly *L. monocytogenes*) and that no net increase in levels of pathogens will occur during cheese production. Requirements are listed for farmers who produce milk for raw milk cheese manufacture. A moving window concept is utilised, in which the last five batches of milk are compared to determine the microbiological quality of raw milk. The NSW Food Authority contends that this approach provides a practical and cost effective means of continuously checking performance (Table 39) and provides early identification of the need for corrective action.

Table 39: NSW recommended raw milk sanitary requirements for raw milk cheese production (using a moving window concept)

Frequency	Criteria	Compliance	
		Target	Upper Limit
Weekly		Four or more batches of the last five must be below this level	No batch of the last five batches may exceed this level
		If a batch fails any of the three criteria above the milk should not be used for raw milk cheese manufacture	
	Bulk Milk Cell Count (SCC)	<200,000 cells/ml (cows' milk)	400,000 cells/ml
		<1,000,000 cells/ml (other species)	No upper limit
	Total Plate Count	<25,000 cfu/ml	50,000 cfu/ml
Routinely	<i>E. coli</i> count	<10 cfu/ml	100 cfu/ml
	<i>S. aureus</i>	<100 cfu/ml	1000 cfu/ml
	<i>L. monocytogenes</i>	Not detected/ 25 ml	NA
	Salmonella	Not detected/ 25 ml	NA

*NA = Not applicable

¹¹⁷ http://www.foodauthority.nsw.gov.au/Documents/licensesandforms/raw_milk_cheese_proforma.pdf

4.2.5 Trend analysis: Physicochemical parameters

The importance of measuring physicochemical parameters and achieving the correct targets was previously discussed in section 2.3.7 of this report. Out-of-specification results, such as cheese that exceeds moisture targets or does not reach pH or salt targets, can lead to microbiological quality issues, with possible implications for product safety if not properly resolved. This particular issue can be easily resolved for example through addition of higher salt levels during production. As noted in section 4.2.6 below, achieving target physicochemical parameters for a given cheese provides evidence of process control, which is essential for achieving microbiological safety.

4.2.6 Case study of physicochemical trend analysis of cheese conducted by the Vermont Institute for Artisan Cheese (VIAC)

Below is a study that was conducted by the Vermont Institute for Artisan Cheese (VIAC) at the University of Vermont. Intense, one-on-one technical assistance was provided to small scale cheese makers in Vermont in 2008 with the goal of developing individual Risk Reduction Protocols for each cheese producer for Risk Reduction Management. Risk Reduction Protocols were developed by conducting on-site visits to each of the participating cheesemakers. The VIAC Technical Team spent a total of four days with each cheese maker.

- On the first visit (Day 1), a comprehensive review of the cheese making process, from milking to ageing, was conducted. This intake process allowed a comprehensive flow sheet of the entire cheese making process and an environmental monitoring plan to be developed. Using a HACCP approach, CCPs in the process were identified. The type of cheese being manufactured, an assessment of risk (high risk to low risk, dependent upon cheese characteristics) and physical notes from the structural facility (condition, layout, traffic flow) were compiled.
- On the second visit (Day 2), cheese making was conducted by the cheese maker with participation from the VIAC technical team. A system for achieving process control was put in place through identification of the key parameters needing routine measurement during cheese making (such as pH, titratable acidity, salt-in-moisture, % moisture) based upon the Federal standard of identity for the cheese being manufactured. Microbiological samples were collected during cheese manufacture. Milk, curds, whey and finished cheese were analysed for SPC, coliforms, somatic cell count (SCC), and for target pathogens consisting of *Listeria monocytogenes*, salmonella, *E. coli* O157:H7 and *Staphylococcus aureus*. Environmental swab and sponge samples were collected from target areas in the cheese manufacturing facility (floor drains, floors, vats, tables, carts, squeegees/floor mops) and analysed for presence of *Listeria monocytogenes*.
- On the third visit (Day 3), data from the microbiological analysis was shared with the cheese maker and recommendations made focusing on critical cheese making areas. The VIAC technical team recommend changes as necessary, such as changes in the make process; physical layout of the facility and reorientation of foot traffic; changes

in sanitation; the need for protective clothing such as gowns, hairnets, gloves, hand washing/ sanitisation; implementation of hygienic zoning; improvements in milk quality etc. The cheese maker determined if and how the recommendations could be implemented. On the fourth visit, cheese making was again conducted with the VIAC technical team and the comprehensive microbiological analysis was again conducted. Data was compared between visit 2 and visit 4 to determine if the recommendations made by the VIAC technical team resulted in risk reduction, improved process control, and improvements in cheese safety and quality.

From this study it was noted Good Manufacturing Practices (GMPs) were lacking in many operations. Producers were asked to review the GMPs and apply necessary changes. One common issue seen across numerous participants was the condition of processing rooms. Most facilities had proper wall coverings but many were not effectively sealed at the base to prevent the entrapment of moisture. Repainting and resealing of joints was also a common recommendation. Rusty equipment and shelves were noted in a few facilities. Rooms were often used for multiple purposes in addition to processing, including packing and storage. Many facilities had cracked and porous concrete floors that can harbour dangerous pathogens such as *L. monocytogenes*. This is a particular risk because these floors are very difficult to clean and sanitise effectively. Compounding this issue was the infrequent cleaning and the use of inadequate tools and/ or chemicals. Advice was provided on proper tools and cleaning schedules. Proper chemical concentrations for individual producers and tasks were provided as very few processors were checking required sanitiser concentration and thus using ineffective concentrations at times. Consultation on how to prepare and check sanitiser concentrations was provided. Another contributing factor to the spread of microorganisms in the facilities visited was the lack of, and/ or improper placement of, sanitising foot baths. Advice was given on proper foot baths, placement, and proper sanitiser concentration. Cheesemaking boots and shoes were also frequently worn outside the processing area without a sufficient sanitary break. Footwear was also rarely cleaned and sanitised on a regular basis. Most importantly, producers were advised on how to alter traffic flow patterns in conjunction with shoe changes, foot baths and cleaning schedules to best prevent cross-contamination. Another common observation was the introduction of items to the cheese vat including pH meters, glass pipettes, cups and human hands and arms. Producers were advised to limit contact with cheese milk/ curds unless the items were properly cleaned and sanitised and did not present a physical hazard.

In addition, a general lack of technical expertise related to the scientific aspects of cheesemaking was observed. Few, if any, producers measured starter culture correctly, or measured any physical or chemical properties of their finished product. Some producers took measurements but did so improperly. Often, pH meters were not calibrated routinely and/ or correctly. Most producers were instructed on how to measure starter culture and measure salt content of cheese. One major observation of concern was the general lack of record keeping. This included the proper use of make

sheets as well as the documentation of temperatures, acidity, lot numbers etc. Individual assistance was provided to each cheese maker on an as needed basis.

The general microbiological quality of raw milk tested through this project indicated adequate hygiene during milk collection and storage. Results that appeared to be out of a normal target range were noted and corrective actions (examples noted below) were shared. *S. aureus* was the most common pathogen isolated from raw milk, whey and curd samples. This is consistent with the findings reported in the scientific literature. This pathogen is an animal health issue that must be treated on farm using standard protocols to control mastitis, including dry antibiotic therapy, stripping foremilk, use of gloves during milking, pre- and post-teat dips and drying teats with paper towels (D'Amico and Donnelly 2010). Each producer was instructed on how to limit the outgrowth of *S. aureus* during cheese manufacture (including recommendations on correct measurement and addition of starter culture to assure optimal acidity development, temperature control and hand sanitation) and what to look for during the manufacture process that may indicate a problem. Future testing protocols were also discussed on numerous farms. No *Listeria* spp. or *Salmonella* spp. were detected in any of the milk samples tested. *E. coli* O157:H7 was isolated from one milk sample as well as the final product manufactured from that milk. As expected *Listeria* spp., including *L. monocytogenes*, were isolated from the environmental swabs collected at numerous facilities, with prevalence rates of 10.7% (D'Amico and Donnelly 2009). Common sites most often included floors and drains and less commonly footwear and items brought in from outside the facility such as milk cans and crates. This information was used to develop proper traffic flow patterns as well as operating procedures to limit cross contamination and producers were provided with corrective actions that included recommendations for rigorous cleaning and disinfection. Data from follow up visits indicated that the corrective actions were effective at controlling the pathogen and preventing reentry and cross contamination.

Representative data from physicochemical analysis from this study is shown in the following three tables (Tables 40, 41 and 42). In the sample blue cheese data, there is notable variation of percentage moisture that was attributed to inconsistent stirring of cheese curd during the make procedure. The higher moisture and low salt in moisture in the batch produced on 9/14 could result in a faster ageing process, shorter shelf life and faster off-flavour development. A batch of Colby (a semi-hard cheese similar to a mild Cheddar) produced on 8/18 exceeded the 40% moisture target established in the U.S. Federal Standards of Identity¹¹⁸ in respect of legal requirements for this cheese type. The low salt in moisture observed in batch 8/18 increases the risk of outgrowth of microbial contaminants, including pathogens. Target salt in moisture for this cheese type is 4.3-4.4%. For the soft ripened cheese, salt values are consistent and fat in dry matter values are on target with the Standard of Identity, as is the salt in moisture, documenting effective process control.

¹¹⁸ <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=133&showFR=1>

Table 40: Physicochemical Analysis: Blue Cheese

	Batch 9/12	Batch 9/14	Batch 9/15
Moisture	37.4%	39.25%	37.96%
Salt	1.97%	1.81%	2.32%
Fat	35%	34%	34%
MNFS	57.54%	59.47%	57.51%
FDM	55.91%	55.97%	54.80%
S/M	5.27%	4.61%	6.11%

MNFS: Moisture non-fat substance FDM: Fat in dry matter S/M: Salt in moisture

Table 41: Physicochemical Analysis: Colby (semi-hard) Cheese

	Batch 8/17	Batch 8/18	Batch 8/19
Moisture	39.16%	40.87%	39.14%
Salt	1.66%	1.03%	1.38%
Fat	31%	31%	32%
MNFS	56.75%	59.23%	57.56%
FDM	50.95%	52.43%	52.58%
S/M	4.24%	2.52%	3.53%

Table 42: Physicochemical Analysis: Soft ripened cheese

	Batch 9/28	Batch 9/30	Batch 10/6
Moisture	48.48%	40.03%	46.83%
Salt	1.64%	1.50%	1.50%
Fat	28%	28.75%	29.5%
MNFS	67.33%	68.81%	66.42%
FDM	54.35%	56.41%	55.48%
S/M	3.38%	3.06%	3.20%

4.2.7 Microbiological trend analysis: Environmental sampling

A follow up project conducted by VIAC¹¹⁹ provided intense one-on-one technical assistance to small scale cheese makers to aid in the development of individual Risk Reduction Protocols. The procedures that were utilised followed those described above, with the following deviations. On the first visit, physical notes from the structural facility (condition, layout, and traffic flow) were compiled. Based on this information an environmental sampling plan comprised of thirty sites was developed to include both food contact (FCS) and non-food contact surfaces (NFCS). Environmental sponge samples were collected from target areas in each cheese manufacturing facility (for example: drains, floors, vats, tables, carts, squeegees, etc.). Upon return to the laboratory, samples were analysed for the presence of *Listeria* species, with a focus on

¹¹⁹ D'Amico, D.J. and C.W. Donnelly (2014). Microbiological Assessment and Intervention to Mitigate Environmental Contamination and *Listeria monocytogenes* Risk in Artisan Cheese Facilities. Journal of Food Protection. 77 (suppl.):189-190

the pathogenic species *Listeria monocytogenes*. When available, milk, curds, whey, brine, water, and/or finished cheese samples were collected for microbiological analysis. These samples were analysed (where applicable) for total aerobic bacteria (aerobic plate count; APC), coliforms and for target pathogens including *Listeria monocytogenes*, salmonella, *E. coli* O157:H7 and *Staphylococcus aureus*. Composition of cheese samples, when provided, was also determined to help inform process control.

The VIAC technical team recommended improvements as necessary (changes in the make process (by improving accuracy with method of, and amount of, starter added, for instance, to control the rate of acidification, change the method of and amount of salt addition etc.), physical layout of the facility and re-orientation of foot traffic, changes in sanitation, implementation of hygienic zoning, improvements in milk quality, etc.). Specific observations and recommendations as well as the results and interpretations of the microbiological analyses were provided along with recommendations to improve product safety and quality. The producer was left to determine if and how the recommendations would be implemented. After allowing producers time to implement changes, a follow-up visit was scheduled. During this on-site follow-up visit, the implementation of recommendations, or the lack thereof, was documented. Using the same approach as the initial on-site visit, a comprehensive microbiological analysis was again conducted to determine if the recommendations made by the VIAC technical team and implementation by the producer resulted in risk reduction, improved process control, and improvements in cheese safety and quality.

The most common issues observed in these artisan cheese facilities were related to cleaning and sanitisation in terms of frequency and efficacy. With the implementation of the U.S. Food Safety Modernization Act (FSMA), sanitation will serve a critical role in preventing the contamination of food. Observations and recommendations concerning cleaning and sanitation will aid producers in proper implementation of sanitation programs. Well-developed and written sanitation standard operating procedures (SOPs) are essential for producers going forward, including the maintenance of related records. Similarly, a general lack of GMP was observed in many operations. Producers were asked to review the current GMPs and apply changes as necessary including constructing written GMP programs.

Another common issue observed across numerous participants was the condition of facilities and most importantly processing rooms. The primary concern noted was cracked and porous concrete floors and wall-floor junctions that can harbour dangerous pathogens such as *L. monocytogenes* because these floors are very difficult to clean and sanitise effectively. Compounding this issue was the infrequent cleaning and the use of inadequate tools and/or chemicals as previously noted. Advice on proper tools and cleaning schedules as well as proper chemical concentrations was provided for individual producers and tasks. For instance, very few processors were checking the concentration of their sanitiser and often using ineffective concentrations. This was a particular issue with chlorine as the efficacy decreases rapidly through the day as it comes into contact with organic matter. Several producers also rinsed sanitiser

immediately following exposure to equipment surfaces thus reducing the effectiveness. This also results in the “re-contamination” of surfaces with microorganisms from the water supply. Most facilities had proper wall coverings but many were not effectively sealed at the base to prevent moisture entrapment, facilitating microbial harborage and growth. This was a particular problem with mould growth. In terms of layout, rooms were often used for multiple purposes in addition to processing, including packing and storage. This results in the presence of physical hazards as well as those of a biological nature. Producers were instructed to limit these items to only those essential to the process and to store others externally to the processing area.

An additional factor contributing to the spread of microorganisms in the facilities visited was footwear that was frequently worn outside the processing area without a sufficient sanitary break. This was most often due to the lack of, or improper placement of sanitising foot baths or other sanitary breaks between rooms. Footwear was also rarely cleaned and sanitised on a regular basis. Advice on proper foot baths, their placement, and proper sanitiser use was provided. Most importantly, producers were advised on how to alter traffic flow patterns in conjunction with shoe changes, foot baths and cleaning schedules to best prevent cross-contamination.

In total, 59 and 87 FCS swabs collected from the initial visits of nine facilities were initially screened for APC and coliforms, respectively, as shown in Table 43 below. From follow-up visits of eight facilities, 52 and 80 samples were analysed for APC and coliforms, respectively. The differences in APC and coliform counts between visits were determined for 35 and 63 resampled FCS sites, respectively. Results indicate an average decrease in APC of 0.64 log cfu/ sponge and a slight increase in coliforms of 0.10 log₁₀ cfu/ sponge.

Table 43: Total aerobic bacteria and coliform counts (log₁₀ cfu/sponge) from food contact surfaces within artisan cheese processing facilities. *SD: Standard deviation. APC: Aerobic plate count.*

	N	Range	Mean (+/- SD)
APC visit 1	59	0 - 8.90	2.52 +/- 1.83
APC visit 2	52	0 - 3.52	1.43 +/- 1
APC visit 2-1	35	-4.82 - 0.98	-0.64 +/- 1.23
Coliforms visit 1	87	0 - 5.09	0.33 +/- 0.95
Coliforms visit 2	80	0 - 3.46	0.21 +/- 0.71
Coliforms (visit 2 - visit 1)	63	-2.86 - 2.26	0.01 +/- 0.77

In total, 30 and 63 NFCS swabs collected from the initial visits of nine facilities were initially screened for total APC and coliforms, respectively (Table 44). As expected, levels were considerably higher than those observed on FCS. From follow-up visits on eight facilities, 15 and 63 samples were analysed for total aerobic bacteria and coliforms, respectively. The differences in APC and coliform counts between visits were determined for 15 and 47 resampled NFCS sites, respectively. Results indicate an

average decrease in APC of 1.62 log cfu/ sponge and a decrease in coliforms of 1.07 log₁₀ cfu/ sponge.

Table 44: Total aerobic bacteria and coliform counts (log₁₀ cfu/sponge) from NFCS within artisan cheese processing facilities. *SD: Standard deviation. APC: Aerobic plate count.*

	N	Range	Mean (+/- SD)
APC visit 1	30	0 - 7.88	3.51 +/- 1.95
APC visit 2	15	0 - 2.46	1.10 +/- 0.83
APC visit 2-1	15	-4.70 - 0.03	-1.62 +/- 1.46
Coliforms visit 1	63	0 - 8.56	2.09 +/- 2.15
Coliforms visit 2	67	0 - 6.2	0.98 +/- 1.58
Coliforms (visit 2 - visit 1)	47	-5.05 - 1.87	-1.07 +/- 1.7

Many facilities had heavy coliform loads on their floors indicating improper separation or sanitary breaks from sources of these organisms, most notably soil and faeces from sites external to the facility. In general, facilities with heavy floor coliform loads had additional contaminated surfaces, especially those close to the floor such as draining table shelves, racks and items stored low on shelves such as floor squeegees constructed of foam, which tend to harbour high coliform loads likely contributing to cross- and recontamination of surfaces. High coliform counts were also noted on surfaces of utensils not thoroughly dried or stacked in a manner impeding drainage and promoting standing water. Equipment with rough welds or other similar niches such as wood handles and hard to reach areas also yielded high coliform levels. This included gaskets, especially in vat outlets, as well as aprons. In most cases, as observed in the reductions achieved between visits, interventions were successful in reducing hygiene indicator bacterial loads.

A total of 165 NFCS sampled during initial visits of the nine facilities were tested for the presence of *Listeria* of which 30 (18.18%) were positive for *Listeria* spp. including 8 (4.85%) positive for *L. monocytogenes*. 10 (6.06%) of these sites, including 6 (3.64%) positive for *L. monocytogenes* were from a single facility that was not sampled again. During follow-up visits at the remaining eight facilities a total of 158 NFCS samples were taken with 10 (6.33%) positive for *Listeria* spp. including 4 (2.53%) positive for *L. monocytogenes*. Common sites sampled included floors and drains and items in contact with floors such as footwear and step stools. Another common contamination source was items brought in from outside the facility, such as the external surfaces of milk cans. This information was used to develop proper traffic flow patterns as well operating procedures to limit cross contamination and producers were provided with corrective actions. Comparison of the incidence from initial assessment to follow-up, documents the elimination of *Listeria* from fifteen previously positive sites including two positive for *L. monocytogenes*. Four of the positive follow-up sites were negative during the initial sampling suggesting possible cross-contamination from other sites or the re-entry of the organism. Six sites were positive in both sampling events suggesting either

recontamination or that current cleaning and sanitation protocols were insufficient to eliminate these organisms. A total of 103 FCS samples during initial visits were tested for the presence of *Listeria* of which only one (0.97%) was positive for *L. monocytogenes*. This sample was from a curd knife. Follow-up sampling documented the elimination of the pathogen following cleaning and sanitation. Overall, all but one facility (89%) had at least one site positive for *Listeria* during at least one visit whereas *L. monocytogenes* was only found in 3 (33%) facilities. In addition to reduction in aerobic bacteria and coliforms, *Listeria* spp. and *L. monocytogenes* contamination rates for NFCS decreased from 18.2% to 6.3%, and 4.9% to 2.5%, respectively.

Results from follow-up visits highlighted the effectiveness of adequate hygiene during milk collection and storage and the relative utility of routine testing. Average APC and coliform counts from initial visits were 12,877 and 131 cfu/ml, respectively. Average levels were substantially lower at follow-up with mean APC and coliform levels of 934 and 4 cfu/ml, respectively.

They examined 14 raw milk samples, 7 (50%) of which were positive for *S. aureus* at a mean level of 70 cfu/ml. Samples from both visits were positive on two farms. In addition to raw milk, four of five curd and whey samples (80%) were also positive for *S. aureus* at average values of 1,543 and 112 cfu/g and ml, respectively. Two of five brine samples (40%) were also positive at average values of 20 cfu/ml. Isolation of *S. aureus* as the most common pathogen in raw milk, whey and curd samples is consistent with findings reported in the scientific literature and previous sampling events. Each producer was instructed on how to limit the outgrowth of *S. aureus* during cheese manufacture (through use of proper starter culture measurement and addition to assure optimal acid production, acidity measurement, proper temperature control and hand sanitation) and what to look for during the manufacture process that may indicate a problem. Future testing protocols were also discussed on numerous farms. *L. monocytogenes* was detected in the milk (mean 17 cfu/ml), curd (mean 11 cfu/g) and whey (mean <1 cfu/ml) from one farm on both visits but not from any other samples. This contamination was preliminarily traced back to the animal feed. No *E. coli* O157:H7 or salmonella were detected in any milk sample.

In most cases, data from follow-up visits detail the elimination of the pathogen from contaminated sites indicating that the corrective actions were often effective at controlling the pathogen and preventing re-entry and cross contamination. In other cases, sufficient changes were not made and this is reflected in the data. The limitations of this work include the fact that this project was conducted during the production season so busy producers explained that they may have intended to make changes but have not had the time necessary. Some sites were not available on both visits so comparative data was not always available. Some samples yielded bacterial loads beyond initial detection limits and therefore had to be estimated, which limits accuracy and comparability and also disqualifies these values from analysis.

S. aureus was the most common organism isolated from in-process samples and brine although *L. monocytogenes* was detected in the milk, whey and resulting cheese from one farm on both visits which was preliminarily traced back to contaminated feed. No *E. coli* O157:H7 or salmonella were detected in any sample.

In conclusion, as in previous risk reduction programs, providing one-on-one technical assistance to cheesemakers through targeted, comprehensive risk reduction visits which involve microbiological data collection is an effective tool to educate cheesemakers about microbiological risks specific to their farm or cheesemaking facility. Our data confirm the value of this type of education, which facilitated decline in incidence and levels of target pathogens and indicator organisms between visits one and two. Such efforts help bring cheesemakers into compliance with regulatory requirements and help cheesemakers protect their products, as well as consumers, from harmful pathogens.

4.2.8 Summary of Recommendations from Chapter 3

To achieve process control that assures consistent production of microbiologically safe and high quality cheeses, cheesemakers must routinely monitor microbiological and physicochemical results from milk and cheese testing. Consistent adherence to SCA recommendations for milk and cheese microbiological targets assures both regulatory compliance and safety. Trend analysis is useful to provide early identification of issues that can be corrected before microbiological problems arise. The SCA recommends that while every batch of cheese or raw material does not need to be tested, cheesemakers should analyse test results obtained over a period of time to identify trends, spikes or other values that deviate from normal. Examples have been provided of systems that facilitate trend analysis, based on cheese risk profiles (DFSV), a moving window concept (Australian NSW Dept. of Primary Industries) or visual analysis through use of five day moving average trend lines. It is also recommended that compositional data, particularly pH and salt-in-moisture values be analysed in a similar way.

Microbiological and physicochemical data taken together should be useful to verify that a HACCP plan is operating as intended. The SCA notes that given the complexity of cheesemaking and the diversity of processes employed, specific recommendations can only be made on a case-by-case basis.

5.0 RECOMMENDATIONS FOR FUTURE WORK

A survey of the microbiological quality of raw milk specifically intended for artisan cheese production in Scotland may provide a baseline from which an assessment of the overall quality of raw milk used for artisan cheese production can be made, and identify areas where improvements can be made. The impact of feeding regimens on microbiological quality of raw milk used for artisan cheese production and effects of feeding dry hay and pasture versus silage and distillers' grains warrants investigation and may reveal sources of contamination that can be mitigated with feed adjustments.

During outbreak investigations, compositional data (e.g. pH, salt and moisture) of cheese involved in outbreaks should be collected. This data could reveal important information regarding causative factors, including lack of process control.

As noted by Lambertini (2015) there is a poor understanding of the dynamics and transmission of STEC virulence in dairy herds and farm environments. The lack of data to support the mathematical modeling of virulence factor spread, persistence, or evolution in farm environments is a major obstacle in the development of predictive tools to assess STEC virulence transmission. More research into this area is recommended. Information in this review also points to the need for good GMP control during milking/storage/transport and good animal husbandry suggesting how to improve these areas and involve farmers to a greater degree would also be worthy of future work.

Providing one-on-one technical assistance to small scale cheese makers to aid in the development of individual Risk Reduction Protocols (including guidance on validation) should be considered.

As consumers demand increased access to locally produced, high quality foods such as artisan cheeses, promoting food safety will be key to sustaining the Scottish artisan cheese industry.

6.0 References

- Abou-Eleinin, A. A., E. T. Ryser, and C. W. Donnelly. 2000. 'Incidence and seasonal variation of *Listeria* species in bulk tank goat's milk', *J Food Prot*, 63: 1208-13.
- Aceto, H., R.J. Munson, D.T. Galligan, C.E. Benson, D. Munro and S. Rankin. . 2000. 'Epidemiological survey of *Salmonella* prevalence in Pennsylvania dairy herds. ', *J. Animal Science*, 78: 107.
- Almena-Aliste, Montserrat, and Bernard Mietton. 2014. 'Cheese Classification, Characterization, and Categorization: A Global Perspective.' in, *Cheese and Microbes* (American Society of Microbiology).
- Altekruse, S. F., B. B. Timbo, J. C. Mowbray, N. H. Bean, and M. E. Potter. 1998. 'Cheese-associated outbreaks of human illness in the United States, 1973 to 1992: sanitary manufacturing practices protect consumers', *J Food Prot*, 61: 1405-7.
- Amato, Ettore, Virginia Filipello, Maria Gori, Sara Lomonaco, Marina Nadia Losio, Antonio Parisi, Pol Huedo, Stephen John Knabel, and Mirella Pontello. 2017. 'Identification of a major *Listeria monocytogenes* outbreak clone linked to soft cheese in Northern Italy – 2009-2011', *BMC Infectious Diseases*, 17: 342.
- Araújo, Valdenice Gomes de, Maria Digian de Oliveira Arruda, Francisca Nayara Dantas Duarte, Janaína Maria Batista de Sousa, Maiara da Costa Lima, Maria Lúcia da Conceição, Donald W. Schaffner, and Evandro Leite de Souza. 2017. 'Predicting and Modelling the Growth of Potentially Pathogenic Bacteria in Coalho Cheese', *J Food Prot*, 80: 1172-81.
- Arimi, Samuel M., Elliot T. Ryser, Todd J. Pritchard, and Catherine W. Donnelly. 1997. 'Diversity of *Listeria* Ribotypes Recovered from Dairy Cattle, Silage, and Dairy Processing Environments', *J Food Prot*, 60: 811-16.
- Bachmann, H. P., and U. Spahr. 1995. 'The fate of potentially pathogenic bacteria in Swiss hard and semihard cheeses made from raw milk', *J Dairy Sci*, 78: 476-83.
- Banks, J.G. 2006. 'Risk Assessment of *L. monocytogenes* in UK retailed Cheese'.
- Bonanno, Ludivine, Benjamin Delubac, Valérie Michel, and Frédéric Auvray. 2017. 'Influence of Stress Factors Related to Cheese-Making Process and to STEC Detection Procedure on the Induction of Stx Phages from STEC O26:H11', *Frontiers in Microbiology*, 8: 296.
- Bone, F. J., D. Bogie, and S. C. Morganjones. 1989. 'Staphylococcal food poisoning from sheep milk cheese', *Epidemiol Infect*, 103: 449-58.
- Boor, K. J. 2005. 'Origin of the 60-day Minimum Holding Period Requirement for United States Cheeses Made from Sub- or Unpasteurized Milk', *Food Prot. Trends*, 25: 767-70.
- Borczyk, A. A., M. A. Karmali, H. Lior, and L. M. Duncan. 1987. 'Bovine reservoir for verotoxin-producing *Escherichia coli* O157:H7', *Lancet*, 1: 98.
- Bradley, R. L., Jr., and M. A. Vanderwarn. 2001. 'Determination of moisture in cheese and cheese products', *J AOAC Int*, 84: 570-92.
- Brusa, Victoria, Virginia Aliverti, Florencia Aliverti, Emanuel E. Ortega, Julian H. de la Torre, Luciano H. Linares, Marcelo E. Sanz, Analía I. Etcheverría, Nora L. Padola, Lucía Galli, Pilar Peral García, Julio Copes, and Gerardo A. Leotta. 2012. 'Shiga toxin-producing *Escherichia coli* in beef retail markets from Argentina', *Frontiers in Cellular and Infection Microbiology*, 2: 171.
- Buazzi, Mahmoud M., Mark E. Johnson, and Elmer H. Marth. 1992. 'Fate of *Listeria monocytogenes* During the Manufacture of Mozzarella Cheese', *J Food Prot*, 55: 80-83.
- Buchanan, Robert L., Leon G. M. Gorris, Melinda M. Hayman, Timothy C. Jackson, and Richard C. Whiting. 2017. 'A review of *Listeria monocytogenes*: An update on outbreaks, virulence, dose-response, ecology, and risk assessments', *Food Control*, 75: 1-13.
- Bula, C. J., J. Bille, and M. P. Glauser. 1995. 'An epidemic of food-borne listeriosis in western Switzerland: description of 57 cases involving adults', *Clin Infect Dis*, 20: 66-72.

- Calicioglu, M. and A. Dikici 2009. 'Survival and Acid Adaptation Ability of Salmonella during Processing and Ripening of Savak tulumu Cheese.', *Journal of Animal and Veterinary Advances*, 8: 1124-30.
- Callaway, T. R., M. A. Carr, T. S. Edrington, R. C. Anderson, and D. J. Nisbet. 2009. 'Diet, *Escherichia coli* O157:H7, and cattle: a review after 10 years', *Curr Issues Mol Biol*, 11: 67-79.
- Cardosa, Patricia, and José Moacir Marin. 2017. 'Occurrence of non-O157 Shiga toxin-encoding *Escherichia coli* in artisanal mozzarella cheese in Brazil: risk factor associated with food workers', *Food Science and Technology*, 37: 41-44.
- Carlton, R. M., W. H. Noordman, B. Biswas, E. D. de Meester, and M. J. Loessner. 2005. 'Bacteriophage P100 for control of *Listeria monocytogenes* in foods: genome sequence, bioinformatic analyses, oral toxicity study, and application', *Regul Toxicol Pharmacol*, 43: 301-12.
- Carminati, D., A. Perrone, E. Neviani, and G. Mucchetti. 2000. 'Influence of traditional brine washing of smear Taleggio cheese on the surface spreading of *Listeria innocua*', *J Food Prot*, 63: 1353-8.
- Charlier, C., M. Cretenet, S. Even, and Y. Le Loir. 2009. 'Interactions between *Staphylococcus aureus* and lactic acid bacteria: An old story with new perspectives', *International Journal of Food Microbiology*, 131: 30-39.
- Chart, H. 2000. 'VTEC enteropathogenicity', *Journal of Applied Microbiology*, 88: 12S-23S.
- Chase-Topping, M., D. Gally, C. Low, L. Matthews, and M. Woolhouse. 2008. 'Super-shedding and the link between human infection and livestock carriage of *Escherichia coli* O157', *Nat Rev Microbiol*, 6: 904-12.
- Choi, Kyoung-Hee, Heeyoung Lee, Soomin Lee, Sejeong Kim, and Yohan Yoon. 2016. 'Cheese Microbial Risk Assessments - A Review', *Asian-Australasian Journal of Animal Sciences*, 29: 307-14.
- Codex. 1978. 'Cdex General Stabdard for Cheese'.
- Cody, S. H., S. L. Abbott, A. A. Marfin, and et al. 1999. 'Two outbreaks of multidrug-resistant salmonella serotype typhimurium dt104 infections linked to raw-milk cheese in northern california', *JAMA*, 281: 1805-10.
- Cremonesi, P., G. Perez, G. Pisoni, P. Moroni, S. Morandi, M. Luzzana, M. Brasca, and B. Castiglioni. 2007. 'Detection of enterotoxigenic *Staphylococcus aureus* isolates in raw milk cheese', *Lett Appl Microbiol*, 45: 586-91.
- Cretenet, Marina, Sergine Even, and Yves Le Loir. 2011. 'Unveiling *Staphylococcus aureus* enterotoxin production in dairy products: a review of recent advances to face new challenges', *Dairy Science & Technology*, 91: 127-50.
- D'Amico, D. J., and C. W. Donnelly. 2009. 'Detection, isolation, and incidence of *Listeria* spp. in small-scale artisan cheese processing facilities: a methods comparison', *J Food Prot*, 72: 2499-507.
- D'Amico, D.J. and C.W. Donnelly. 2010. 'Microbiological quality of raw milk used for small-scale artisan cheese production in Vermont: effect of farm characteristics and practices', *J Dairy Sci*, 93: 134-47.
- D'Amico, D. J., M. J. Druart, and C. W. Donnelly. 2008. '60-day aging requirement does not ensure safety of surface-mold-ripened soft cheeses manufactured from raw or pasteurized milk when *Listeria monocytogenes* is introduced as a postprocessing contaminant', *J Food Prot*, 71: 1563-71.
- D'Amico, D. J., M. J. Druart, and C. W. Donnelly . 2010. 'Behavior of *Escherichia coli* O157:H7 during the manufacture and aging of Gouda and stirred-curd Cheddar cheeses manufactured from raw milk', *J Food Prot*, 73: 2217-24.

- D'Amico, D. J., M. J. Druart, and C. W. Donnelly 2014. 'Comparing the behavior of multidrug-resistant and pansusceptible *Salmonella* during the production and aging of a Gouda cheese manufactured from raw milk', *J Food Prot*, 77: 903-13.
- D'Amico, D. J., E. Groves, and C. W. Donnelly. 2008. 'Low incidence of foodborne pathogens of concern in raw milk utilized for farmstead cheese production', *J Food Prot*, 71: 1580-9.
- D'Amico, Dennis J., and Catherine W. Donnelly. 2011. 'Characterization of *Staphylococcus aureus* Strains Isolated from Raw Milk Utilized in Small-Scale Artisan Cheese Production', *J Food Prot*, 74: 1353-58.
- D'Amico, Dennis, and Catherine W. Donnelly. 2017. 'Chapter 22 - Growth and Survival of Microbial Pathogens in Cheese.' in, *Cheese (Fourth edition)* (Academic Press: San Diego).
- D'Amico, Dennis J., and Catherine W. Donnelly. 2011. 'FDA's domestic and imported cheese compliance program results: January 1, 2004–December 31, 2006. ', *Food Protection Trends*, 31: 216-26.
- Dalmasso, Marion, and Kieran Jordan. 2013. 'Absence of growth of *Listeria monocytogenes* in naturally contaminated Cheddar cheese', *Journal of Dairy Research*, 81: 46-53.
- De Buyser, Marie-Laure, Barbara Dufour, Murielle Maire, and Véronique Lafarge. 2001. 'Implication of milk and milk products in food-borne diseases in France and in different industrialised countries', *International Journal of Food Microbiology*, 67: 1-17.
- De Reu, K., K. Grijspeerdt, and L. Herman. 2004. 'A Belgian survey of hygiene indicator bacteria and pathogenic bacteria in raw milk and direct marketing of raw milk farm products', *Journal of Food Safety*, 24: 17-36.
- Delbes, C., J. Alomar, N. Chougui, J. F. Martin, and M. C. Montel. 2006. '*Staphylococcus aureus* growth and enterotoxin production during the manufacture of uncooked, semihard cheese from cows' raw milk', *J Food Prot*, 69: 2161-7.
- Dewell, G.A., J.R. Ransom, R.D. Dewell, K. McCurdy, I.A. Gardner, E. Hill, J.N. Sofos, K.E. Belk, G.C. Smith, and M.D. Salman. 2005. 'Prevalence of and Risk Factors for *Escherichia coli* O157 in Market-Ready Beef Cattle from 12 U.S. Feedlots', *Foodborne Pathogens and Disease*, 2: 70-76.
- Diez-Gonzalez, Francisco, Todd R. Callaway, Menas G. Kizoulis, and James B. Russell. 1998. 'Grain Feeding and the Dissemination of Acid-Resistant *Escherichia coli* from Cattle', *Science*, 281: 1666-68.
- Dominguez, M., N. Jourdan-Da Silva, V. Vaillant, N. Pihier, C. Kermin, F. X. Weill, G. Delmas, A. Kerouanton, A. Brisabois, and H. de Valk. 2009. 'Outbreak of *Salmonella enterica* serotype Montevideo infections in France linked to consumption of cheese made from raw milk', *Foodborne Pathog Dis*, 6: 121-8.
- Donnelly, C. 2001. 'Factors associated with hygienic control and quality of cheeses prepared from raw milk: a review.', *Bull. Int. Dairy Fed.*, 369: 16-27.
- Donnelly, C. W. 2004. 'Growth and Survival of Microbial Pathogens in Cheese.' in Patrick F. Fox, Paul L. H. McSweeney, Timothy M. Cogan and Timothy P. Guinee (eds.), *Cheese: Chemistry, Physics and Microbiology* (Academic Press).
- Doran, Kelly S., Anirban Banerjee, Olivier Disson, and Marc Lecuit. 2013. 'Concepts and Mechanisms: Crossing Host Barriers', *Cold Spring Harbor Perspectives in Medicine*, 3: a010090.
- Doyle, C. J., D. Gleeson, P. W. O'Toole, and P. D. Cotter. 2017. 'Impacts of Seasonal Housing and Teat Preparation on Raw Milk Microbiota: a High-Throughput Sequencing Study', *Appl Environ Microbiol*, 83.
- Driehuis, F. 2013. 'Silage and the safety and quality of dairy foods: a review', *Agricultural and Food Science*, 22: 16-34.

- Duffy, G., R. C. Whiting, and J. J. Sheridan. 1999. 'The effect of a competitive microflora, pH and temperature on the growth kinetics of *Escherichia coli* O157:H7', *Food Microbiology*, 16: 299-307.
- El-Gazzar, Fathy E., and Elmer H. Marth. 1992. 'Salmonellae, Salmonellosis, and Dairy Foods: A Review', *Journal of Dairy Science*, 75: 2327-43.
- Erickson, M. C., and M. P. Doyle. 2007. 'Food as a vehicle for transmission of Shiga toxin-producing *Escherichia coli*', *J Food Prot*, 70: 2426-49.
- Espie, E., V. Vaillant, P. Mariani-Kurkdjian, F. Grimont, R. Martin-Schaller, H. De Valk, and C. Vernozy-Rozand. 2006. '*Escherichia coli* O157 outbreak associated with fresh unpasteurized goats' cheese', *Epidemiol Infect*, 134: 143-46.
- European Food Safety, Authority. 2013. 'Analysis of the baseline survey on the prevalence of *Listeria monocytogenes* in certain ready-to-eat foods in the EU, 2010–2011 Part A: *Listeria monocytogenes* prevalence estimates', *EFSA Journal*, 11: 3241-n/a.
- FACEnetwork. 2016. "European Guide for Good Hygiene Practices in the production of artisanal cheese and dairy products." In, edited by Farmhouse and Artisan Cheese and Dairy Producers European Network.
- Farber, J. M. 1990. 'Listeriosis Traced to the Consumption of Alfalfa Tablets and Soft Cheese', *New England Journal of Medicine*, 322: 338-38.
- Farber, J. M., and P. I. Peterkin. 1991a. '*Listeria monocytogenes*, a food-borne pathogen', *Microbiological Reviews*, 55: 476-511.
- Farber, J. M., and P. I. Peterkin 1991b. '*Listeria monocytogenes*, a food-borne pathogen', *Microbiol Rev*, 55: 476-511.
- Farrokh, Choreh, Kieran Jordan, Frederic Auvray, Kathleen Glass, Hanne Oppegaard, Sabrina Raynaud, Delphine Thevenot, Robin Condron, Koen De Reu, Alexander Govaris, Klaus Heggum, Marc Heyndrickx, Joerg Hummerjohann, Denise Lindsay, Stephane Miszczycha, Sylvie Moussiegt, Karen Verstraete, and Olivier Cerf. 2013. 'Review of Shiga-toxin-producing *Escherichia coli* (STEC) and their significance in dairy production', *International Journal of Food Microbiology*, 162: 190-212.
- FDA. 2003. 'Quantitative Assessment of Relative Risk to Public Health From Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods '.
- FDA. 2011.
- FDA. 2016. 'FY 2014 – 2016 Microbiological Sampling Assignment Summary Report: Raw Milk Cheese Aged 60 Days'.
- FDA, Health Canada. 2015. 'Joint FDA / Health Canada Quantitative Assessment of the Risk of Listeriosis from Soft-Ripened Cheese Consumption in the United States and Canada: Report. '.
- Ferreira, V., M. Wiedmann, P. Teixeira, and M. J. Stasiewicz. 2014. '*Listeria monocytogenes* Persistence in Food-Associated Environments: Epidemiology, Strain Characteristics, and Implications for Public Health', *J Food Prot*, 77: 150-70.
- Ferrier, Rachel, Bernard Hezard, Adrienne Lintz, Valérie Stahl, and Jean-Christophe Augustin. 2013. 'Combining Individual-Based Modeling and Food Microenvironment Descriptions To Predict the Growth of *Listeria monocytogenes* on Smear Soft Cheese', *Appl Environ Microbiol*, 79: 5870-81.
- Fister, Susanne, Christian Robben, Anna K. Witte, Dagmar Schoder, Martin Wagner, and Peter Rossmannith. 2016. 'Influence of Environmental Factors on Phage–Bacteria Interaction and on the Efficacy and Infectivity of Phage P100', *Frontiers in Microbiology*, 7.
- Fontaine, R. E., M. L. Cohen, W. T. Martin, and T. M. Vernon. 1980. 'Epidemic salmonellosis from cheddar cheese: surveillance and prevention', *Am J Epidemiol*, 111: 247-53.
- Fox, E., K. Hunt, M. O'Brien, and K. Jordan. 2011. '*Listeria monocytogenes* in Irish Farmhouse cheese processing environments', *Int J Food Microbiol*, 145 Suppl 1: S39-45.

- Fox, Patrick F., Timothy P. Guinee, Timothy M. Cogan, and Paul L. H. McSweeney. 2017. 'Pathogens in Cheese and Foodborne Illnesses.' in, *Fundamentals of Cheese Science* (Springer US: Boston, MA).
- FSIA. 2015. 'Raw milk and raw milk filter microbiological surveillance programme', *Monitoring and Surveillance Series: Microbiology*; 12NS2.
- Fuka, Mirna Mrkonjić, Stefanie Wallisch, Marion Engel, Gerhard Welzl, Jasmina Havranek, and Michael Schlöter. 2013. 'Dynamics of Bacterial Communities during the Ripening Process of Different Croatian Cheese Types Derived from Raw Ewe's Milk Cheeses', *PLoS ONE*, 8: e80734.
- Gal-Mor, Ohad, Erin C. Boyle, and Guntram A. Grassl. 2014. 'Same species, different diseases: how and why typhoidal and non-typhoidal *Salmonella enterica* serovars differ', *Frontiers in Microbiology*, 5: 391.
- Garage, Shantini D., Angela K. Patton, James F. Hanson, and Alison A. Weiss. 2004. 'Diversity and Host Range of Shiga Toxin-Encoding Phage', *Infection and Immunity*, 72: 7131-39.
- Gaulin, C., D. Ramsay, and S. Bekal. 2012. 'Widespread listeriosis outbreak attributable to pasteurized cheese, which led to extensive cross-contamination affecting cheese retailers, Quebec, Canada, 2008', *J Food Prot*, 75: 71-8.
- Gay, Marielle, and Albert Amgar. 2005. 'Factors moderating *Listeria monocytogenes* growth in raw milk and in soft cheese made from raw milk', *Lait*, 85: 153-70.
- Genigeorgis, C. A. 1989. 'Present state of knowledge on *staphylococcal* intoxication', *Int J Food Microbiol*, 9: 327-60.
- Gilman, H. L., A. C. Dahlberg, and J. C. Marquardt. 1946. 'The Occurrence and Survival of *Brucella Abortus* in Cheddar and Limburger Cheese*', *Journal of Dairy Science*, 29: 71-85.
- Goepfert, J. M., N. F. Olson, and E. H. Marth. 1968. 'Behavior of *Salmonella* Typhimurium during manufacture and curing of cheddar cheese', *Appl Microbiol*, 16: 862-6.
- Gould, L. H., E. Mungai, and C. B. Behraves. 2014. 'Outbreaks attributed to cheese: differences between outbreaks caused by unpasteurized and pasteurized dairy products, United States, 1998-2011', *Foodborne Pathog Dis*, 11: 545-51.
- Guenther, S., and M. J. Loessner. 2011. 'Bacteriophage biocontrol of *Listeria monocytogenes* on soft ripened white mold and red-smear cheeses', *Bacteriophage*, 1: 94-100.
- Gurtler, Joshua B., and Jeffrey L. Kornacki. 2007. 'Incidence and Control of *Listeria* in Food Processing Facilities.' in, *Listeria, Listeriosis, and Food Safety, Third Edition* (CRC Press).
- Hammer, P., W. Bockelmann, and W. Hoffmann. 2017. 'Fate of *Listeria innocua* during production and ripening of smeared hard cheese made from raw milk', *J Dairy Sci*.
- Hargrove, R.E., F. E. McDonough, and W. A. Mattingly. 1969. 'Factors affecting survival of salmonella in cheddar and colby cheese', *Journal of Milk and Food Technology*, 32: 480-84.
- Harter, E., E. M. Wagner, A. Zaiser, S. Halecker, M. Wagner, and K. Rychli. 2017. 'Stress Survival Islet 2, Predominantly Present in *Listeria monocytogenes* Strains of Sequence Type 121, Is Involved in the Alkaline and Oxidative Stress Responses', *Appl Environ Microbiol*, 83.
- Heiman, K. E., V. B. Garalde, M. Gronostaj, K. A. Jackson, S. Beam, L. Joseph, A. Saupe, E. Ricotta, H. Waechter, A. Wellman, M. Adams-Cameron, G. Ray, A. Fields, Y. Chen, A. Datta, L. Burall, A. Sabol, Z. Kucerova, E. Trees, M. Metz, P. Leblanc, S. Lance, P. M. Griffin, R. V. Tauxe, and B. J. Silk. 2016. 'Multistate outbreak of listeriosis caused by imported cheese and evidence of cross-contamination of other cheeses, USA, 2012', *Epidemiol Infect*, 144: 2698-708.
- Hennekinne, Jacques-Antoine, Martine Gohier, Tiphaine Maire, Christiane Lapeyre, Bertrand Lombard, and Sylviane Dragacci. 2003. *First Proficiency Testing To Evaluate the Ability*

of European Union National Reference Laboratories To Detect Staphylococcal Enterotoxins in Milk Products.

- Henry, M. K., S. C. Tongue, J. Evans, C. Webster, Kendrick Ij Mc, M. Morgan, A. Willett, A. Reeves, R. W. Humphry, D. L. Gally, G. J. Gunn, and M. E. Chase-Topping. 2017. 'British *Escherichia coli* O157 in Cattle Study (BECS): to determine the prevalence of *E. coli* O157 in herds with cattle destined for the food chain', *Epidemiol Infect*, 145: 3168-79.
- Honish, L., G. Predy, N. Hislop, L. Chui, K. Kowalewska-Grochowska, L. Trottier, C. Kreplin, and I. Zazulak. 2005. 'An outbreak of *E. coli* O157 : H7 hemorrhagic colitis associated with unpasteurized gouda cheese', *Canadian Journal of Public Health-Revue Canadienne De Sante Publique*, 96: 182-84.
- Ingham, S. C., Y. C. Su, and D. S. Spangenberg. 2000. 'Survival of *Salmonella* Typhimurium and *Escherichia coli* O157:H7 in cheese brines', *Int J Food Microbiol*, 61: 73-9.
- Irlinger, F., S. Layec, S. Helinck, and E. Dugat-Bony. 2015. 'Cheese rind microbial communities: diversity, composition and origin', *FEMS Microbiol Lett*, 362: 1-11.
- Jaakkonen, A., S. Salmenlinna, R. Rimhanen-Finne, H. Lundström, S. Heinikainen, M. Hakkinen, and S. Hallanvuo. 2017. 'Severe Outbreak of Sorbitol-Fermenting *Escherichia coli* O157 via Unpasteurized Milk and Farm Visits, Finland 2012', *Zoonoses and Public Health*, 64: 468-75.
- Jacob, M. E., J. T. Fox, J. S. Drouillard, D. G. Renter, and T. G. Nagaraja. 2008. 'Effects of Dried Distillers' Grain on Fecal Prevalence and Growth of *Escherichia coli* O157 in Batch Culture Fermentations from Cattle', *Appl Environ Microbiol*, 74: 38-43.
- Jacquet, C., J. Rocourt, and A. Reynaud. 1993. 'Study of *Listeria monocytogenes* contamination in a dairy plant and characterization of the strains isolated', *Int J Food Microbiol*, 20: 13-22.
- Jasson, V., A. Rajkovic, L. Baert, J. Debevere, and M. Uyttendaele. 2009. 'Comparison of enrichment conditions for rapid detection of low numbers of sublethally injured *Escherichia coli* O157 in food', *J Food Prot*, 72: 1862-8.
- Jayarao, B. M., and D. R. Henning. 2001. 'Prevalence of foodborne pathogens in bulk tank milk', *J Dairy Sci*, 84: 2157-62.
- Johnson, Eric A., John H. Nelson, and Mark Johnson. 1990. 'Microbiological Safety of Cheese Made from Heat-Treated Milk, Part II. Microbiology', *J Food Prot*, 53: 519-40.
- Johnson, Mark E., and N. F. Olson. 1985. 'A Comparison of Available Methods for Determining Salt Levels in Cheese', *Journal of Dairy Science*, 68: 1020-24.
- Jørgensen, H. J., T. Mørk, and L. M. Rørvik. 2005. 'The Occurrence of *Staphylococcus aureus* on a Farm with Small-Scale Production of Raw Milk Cheese', *Journal of Dairy Science*, 88: 3810-17.
- Kadariya, Jhalka, Tara C. Smith, and Dipendra Thapaliya. 2014. '*Staphylococcus aureus* and Staphylococcal Food-Borne Disease: An Ongoing Challenge in Public Health', *BioMed Research International*, 2014: 827965.
- Kasrazadeh, M., and C. Genigeorgis. 1995. 'Potential growth and control of *Escherichia coli* O157:H7 in soft hispanic type cheese', *Int J Food Microbiol*, 25: 289-300.
- Kindstedt, P. 2005. 'American farmstead cheese : the complete guide to making and selling artisan cheeses '.
- Kindstedt, P. S., and F. V. Kosikowski. 1984. 'Measurement of Sodium Chloride in Cheese by a Simple Sodium Ion Electrode Method', *Journal of Dairy Science*, 67: 879-83.
- King, N., R. Lake, P. Cressey and A. Hudson. 2014. 'Risk profile: shiga toxin-producing *Escherichia coli* in raw milk', *Ministry for Primary Industries*.
- Kocharunchitt, C. and T. Ross. 2015. 'Quantitative assessment of microbiological safety of raw milk cheeses manufacturing ': 1-159.

- Konowalchuk, J., J. I. Speirs, and S. Stavric. 1977. 'Vero response to a cytotoxin of *Escherichia coli*', *Infection and Immunity*, 18: 775-79.
- Kousta, Maria, Marios Mataragas, Panagiotis Skandamis, and Eleftherios H. Drosinos. 2010. 'Prevalence and sources of cheese contamination with pathogens at farm and processing levels', *Food Control*, 21: 805-15.
- Kremer, P. H. C., J. A. Lees, M. M. Koopmans, B. Ferwerda, A. W. M. Arends, M. M. Feller, K. Schipper, M. Valls Seron, A. van der Ende, M. C. Brouwer, D. van de Beek, and S. D. Bentley. 2017. 'Benzalkonium tolerance genes and outcome in *Listeria monocytogenes* meningitis', *Clinical Microbiology and Infection*, 23: 265.e1-65.e7.
- Lambertini, E., J. S. Karns, J. A. Van Kessel, H. Cao, Y. H. Schukken, D. R. Wolfgang, J. M. Smith, and A. K. Pradhan. 2015. 'Dynamics of *Escherichia coli* Virulence Factors in Dairy Herds and Farm Environments in a Longitudinal Study in the United States', *Appl Environ Microbiol*, 81: 4477-88.
- Lambertz, S. Thisted, C. Nilsson, A. Brådenmark, S. Sylvén, A. Johansson, Lisa-Marie Jansson, and M. Lindblad. 2012. 'Prevalence and level of *Listeria monocytogenes* in ready-to-eat foods in Sweden 2010', *International Journal of Food Microbiology*, 160: 24-31.
- Le Loir, Y., F. Baron, and M. Gautier. 2003. '*Staphylococcus aureus* and food poisoning', *Genet Mol Res*, 2: 63-76.
- Lekkas, P. and C.W. Donnelly. 2016. 'Farm sources of *Listeria monocytogenes* and impact on the microbial safety of milk destined for artisan cheese production', *P2-141 IAFP Annual Meeting Abstracts*.
- Lester, A., B. G. Bruun, P. Husum, H. J. Kolmos, B. B. Nielsen, J. H. Scheibel, N. Skovgaard, and F. Thune-Stephensen. 1995. '[*Salmonella* Dublin]', *Ugeskr Laeger*, 157: 20-4.
- Leyer, G J, and E A Johnson. 1992. 'Acid adaptation promotes survival of *Salmonella* spp. in cheese', *Appl Environ Microbiol*, 58: 2075-80.
- Lina, Gerard, Gregory A. Bohach, Sean P. Nair, Keiichi Hiramatsu, Evelyn Jouvin-Marche, and Roy Mariuzza. 2004. 'Standard Nomenclature for the Superantigens Expressed by *Staphylococcus*', *The Journal of Infectious Diseases*, 189: 2334-36.
- Lindblad, M., and R. Lindqvist. 2010. 'Modelling time to growth of *Escherichia coli* as a function of water activity and undissociated lactic acid', *Letters in Applied Microbiology*, 50: 308-13.
- Linnan , Michael J., Laurene Mascola , Xiao Dong Lou , Veronique Goulet , Susana May , Carol Salminen , David W. Hird , M. Lynn Yonekura , Peggy Hayes , Robert Weaver , Andre Audurier , Brian D. Plikaytis , Shirley L. Fannin , Abraham Kleks , and Claire V. Broome 1988. 'Epidemic Listeriosis Associated with Mexican-Style Cheese', *New England Journal of Medicine*, 319: 823-28.
- Little, C. L., J. R. Rhoades, S. K. Sagoo, J. Harris, M. Greenwood, V. Mithani, K. Grant, and J. McLauchlin. 2008. 'Microbiological quality of retail cheeses made from raw, thermized or pasteurized milk in the UK', *Food Microbiology*, 25: 304-12.
- Locking, M, L Allison, L Rae, Kevin Pollock, and M Hanson. 2006. 'VTEC infections and livestock-related exposures in Scotland, 2004', *Weekly releases (1997–2007)*, 11: 2908.
- Locking, Mary E., Kevin G. J. Pollock, Lesley J. Allison, Linda Rae, Mary F. Hanson, and John M. Cowden. 2011. '*Escherichia coli* O157 Infection and Secondary Spread, Scotland, 1999-2008', *Emerging Infectious Diseases*, 17: 524-27.
- Lovett, J., D. W. Francis, and J. M. Hunt. 1987. '*Listeria monocytogenes* in Raw Milk: Detection, Incidence, and Pathogenicity', *J Food Prot*, 50: 188-92.
- Madic, J., N. Vingadassalon, C. P. de Garam, M. Marault, F. Scheutz, H. Brugere, E. Jamet, and F. Auvray. 2011. 'Detection of Shiga toxin-producing *Escherichia coli* serotypes O26:H11, O103:H2, O111:H8, O145:H28, and O157:H7 in raw-milk cheeses by using multiplex real-time PCR', *Appl Environ Microbiol*, 77: 2035-41.

- Maguire, H. C. F., M. Boyle, M. J. Lewis, J. Pankhurst, A. A. Wieneke, M. Jacob, J. Bruce, and M. Omahony. 1991. 'A large outbreak of food poisoning of unknown etiology associated with stilton cheese', *Epidemiol Infect*, 106: 497-505.
- Maguire, H., J. Cowden, M. Jacob, B. Rowe, D. Roberts, J. Bruce, and E. Mitchell. 1992. 'An Outbreak of *Salmonella* Dublin Infection in England and Wales Associated with a Soft Unpasteurized Cows' Milk Cheese', *Epidemiol Infect*, 109: 389-96.
- Maher, M. M., K. N. Jordan, M. E. Upton, and A. Coffey. 2001. 'Growth and survival of *E. coli* O157:H7 during the manufacture and ripening of a smear-ripened cheese produced from raw milk', *J Appl Microbiol*, 90: 201-7.
- Marek, Patrick, Manoj Kumar Mohan Nair, Thomas Hoagland, and Kumar Venkitanarayanan. 2004. 'Survival and growth characteristics of *Escherichia coli* O157:H7 in pasteurized and unpasteurized Cheddar cheese whey', *International Journal of Food Microbiology*, 94: 1-7.
- Marozzi, Selene, Paola De Santis, Sarah Lovari, Roberto Condoleo, Stefano Bilei, Rita Marcianò, and Ziad Mezher. 2016. 'Prevalence and Molecular Characterisation of Shiga Toxin-Producing *Escherichia coli* in Raw Milk Cheeses from Lazio Region, Italy', *Italian Journal of Food Safety*, 5: 4566.
- Martin, M. L., L. D. Shipman, J. G. Wells, M. E. Potter, K. Hedberg, I. K. Wachsmuth, R. V. Tauxe, J. P. Davis, J. Arnoldi, and J. Tilleli. 1986. 'Isolation of *Escherichia coli* O157:H7 from dairy cattle associated with two cases of haemolytic uraemic syndrome', *Lancet*, 2: 1043.
- Matthews, Louise, Richard Reeve, David L. Gally, J. Chris Low, Mark E. J. Woolhouse, Sean P. McAteer, Mary E. Locking, Margo E. Chase-Topping, Daniel T. Haydon, Lesley J. Allison, Mary F. Hanson, George J. Gunn, and Stuart W. J. Reid. 2013. 'Predicting the public health benefit of vaccinating cattle against *Escherichia coli* O157', *Proceedings of the National Academy of Sciences of the United States of America*, 110: 16265-70.
- McCollum, J. T., N. J. Williams, S. W. Beam, S. Cosgrove, P. J. Ettestad, T. S. Ghosh, A. C. Kimura, L. Nguyen, S. G. Stroika, R. L. Vogt, A. K. Watkins, J. R. Weiss, I. T. Williams, and A. B. Cronquist. 2012. 'Multistate Outbreak of *Escherichia coli* O157:H7 Infections Associated with In-Store Sampling of an Aged Raw-Milk Gouda Cheese, 2010', *J Food Prot*, 75: 1759-65.
- Miszczyscha, Stephane D., Frederique Perrin, Sarah Ganet, Emmanuel Jamet, Fanny Tenenhaus-Aziza, Marie-Christine Montel, and Delphine Thevenot-Sergentet. 2013. 'Behavior of Different Shiga Toxin-Producing *Escherichia coli* Serotypes in Various Experimentally Contaminated Raw-Milk Cheeses', *Appl Environ Microbiol*, 79: 150-58.
- Montel, Marie-Christine, Solange Buchin, Adrien Mallet, Céline Delbes-Paus, Dominique A. Vuitton, Nathalie Desmasures, and Françoise Berthier. 2014. 'Traditional cheeses: Rich and diverse microbiota with associated benefits', *International Journal of Food Microbiology*, 177: 136-54.
- Montet, M. P., E. Jamet, S. Ganet, M. Dizin, S. Miszczycha, L. Duniere, D. Thevenot, and C. Vernozy-Rozand. 2009. 'Growth and Survival of Acid-Resistant and Non-Acid-Resistant Shiga-Toxin-Producing *Escherichia coli* Strains during the Manufacture and Ripening of Camembert Cheese', *Int J Microbiol*, 2009: 653481.
- Morgan, D., C. P. Newman, D. N. Hutchinson, A. M. Walker, B. Rowe, and F. Majid. 1993. 'Verotoxin producing *Escherichia coli* O 157 infections associated with the consumption of yoghurt', *Epidemiol Infect*, 111: 181-87.
- MPI. 2015. 'Quantitative Assessment of Microbiological Safety of Raw Milk Cheeses Manufacturing'.
- Munns, K. D., L. B. Selinger, K. Stanford, L. Guan, T. R. Callaway, and T. A. McAllister. 2015. 'Perspectives on super-shedding of *Escherichia coli* O157:H7 by cattle', *Foodborne Pathog Dis*, 12: 89-103.

- Murphy, Brenda P., Evonne McCabe, Mary Murphy, James F. Buckley, Dan Crowley, Séamus Fanning, and Geraldine Duffy. 2016. 'Longitudinal Study of Two Irish Dairy Herds: Low Numbers of Shiga Toxin-Producing *Escherichia coli* O157 and O26 Super-Shedders Identified', *Frontiers in Microbiology*, 7: 1850.
- NACMCF. 2006. 'Requisite Scientific Parameters for Establishing the Equivalence of Alternative Methods of Pasteurization', *J Food Prot*, 69: 1190-216.
- NACMCF 2010. 'Parameters for Determining Inoculated Pack/Challenge Study Protocols', *J Food Prot*, 73: 140-202.
- Neves, E., A. C. Silva, S. M. Roche, P. Velge, and L. Brito. 2008. 'Virulence of *Listeria monocytogenes* isolated from the cheese dairy environment, other foods and clinical cases', *J Med Microbiol*, 57: 411-5.
- Nightingale, K. K., Y. H. Schukken, C. R. Nightingale, E. D. Fortes, A. J. Ho, Z. Her, Y. T. Grohn, P. L. McDonough, and M. Wiedmann. 2004. 'Ecology and Transmission of *Listeria monocytogenes* Infecting Ruminants and in the Farm Environment', *Appl Environ Microbiol*, 70: 4458-67.
- Nobili, G., I. Franconieri, M. G. Basanisi, G. La Bella, R. Tozzoli, A. Caprioli, and G. La Salandra. 2016. 'Short communication: Isolation of Shiga toxin-producing *Escherichia coli* in raw milk and mozzarella cheese in southern Italy', *J Dairy Sci*, 99: 7877-80.
- O'Donnell, E. T. 1995. 'The incidence of *Salmonella* and *Listeria* in raw milk from farm bulk tanks in England and Wales', *International Journal of Dairy Technology*, 48: 25-29.
- Omisakin, F., M. MacRae, I. D. Ogden, and N. J. C. Strachan. 2003. 'Concentration and Prevalence of *Escherichia coli* O157 in Cattle Feces at Slaughter', *Appl Environ Microbiol*, 69: 2444-47.
- Ooi, Say Tat, and Bennett Lorber. 2005. 'Gastroenteritis Due to *Listeria monocytogenes*', *Clinical Infectious Diseases*, 40: 1327-32.
- Orsi, Renato H., Henk C. den Bakker, and Martin Wiedmann. 2011. '*Listeria monocytogenes* lineages: Genomics, evolution, ecology, and phenotypic characteristics', *International Journal of Medical Microbiology*, 301: 79-96.
- Orsi, Renato H., Mark L. Borowsky, Peter Lauer, Sarah K. Young, Chad Nusbaum, James E. Galagan, Bruce W. Birren, Reid A. Ivy, Qi Sun, Lewis M. Graves, Bala Swaminathan, and Martin Wiedmann. 2008. 'Short-term genome evolution of *Listeria monocytogenes* in a non-controlled environment', *BMC Genomics*, 9: 539-39.
- Ortenzi, Roberta, Raffaella Branciarri, Sara Primavilla, David Ranucci, and Andrea Valiani. 2015. 'Behaviour of *Listeria Monocytogenes* in Artisanal Raw Milk Pecorino Umbro Cheese: A Microbiological Challenge Test', *Italian Journal of Food Safety*, 4: 5370.
- Ostergaard, N. B., A. Eklow, and P. Dalgaard. 2014. 'Modelling the effect of lactic acid bacteria from starter- and aroma culture on growth of *Listeria monocytogenes* in cottage cheese', *Int J Food Microbiol*, 188: 15-25.
- Pan, Y., F. Breidt, and S. Kathariou. 2006. 'Resistance of *Listeria monocytogenes* Biofilms to Sanitizing Agents in a Simulated Food Processing Environment', *Appl Environ Microbiol*, 72: 7711-17.
- Papadopoulou, C., Maipa, V., Dimitriou, D., Pappas, C., Voutsinas, L., And Malatou, H (1993) Behavior of *Salmonella enteritidis* During the Manufacture, Ripening, and Storage of Feta Cheese Made from Unpasteurized Ewe's Milk. *Journal of Food Protection*: January 1993, Vol. 56, No. 1, pp. 25-28. <https://doi.org/10.4315/0362-028X-56.1.25>
- Park, H. S., E. H. Marth, J. M. Goepfert, and N. F. Olson. 1970. 'The fate of *Salmonella* Typhimurium in the manufacture and ripening of low-acid cheddar cheese', *Journal of Milk and Food Technology*, 33: 280-84.
- Pennington, Thomas Hugh. 2014. '*E. coli* O157 outbreaks in the United Kingdom: past, present, and future', *Infection and Drug Resistance*, 7: 211-22.

- Perrin, Frédérique, Fanny Tenenhaus-Aziza, Valérie Michel, Stéphane Mischczycha, Nadège Bel, and Moez Sanaa. 2015. 'Quantitative Risk Assessment of Haemolytic and Uremic Syndrome Linked to O157:H7 and Non-O157:H7 Shiga-Toxin Producing *Escherichia coli* Strains in Raw Milk Soft Cheeses', *Risk Analysis*, 35: 109-28.
- Pichler, Juliane, Georg Appl, Ariane Pietzka, and Franz Allerberger. 2011. 'Lessons to be learned from an outbreak of foodborne Listeriosis, Austria 2009–2010', *Food Protection Trends*, 31: 268-73.
- Pritchard, Todd J., Constance M. Beliveau, Kathy J. Flanders, and Catherine W. Donnelly. 1994. 'Increased Incidence of *Listeria* Species in Dairy Processing Plants Having Adjacent Farm Facilities', *J Food Prot*, 57: 770-75.
- Quigley, Lisa, Orla O'Sullivan, Catherine Stanton, Tom P. Beresford, R. Paul Ross, Gerald F. Fitzgerald, and Paul D. Cotter. 2013. 'The complex microbiota of raw milk', *FEMS Microbiology Reviews*, 37: 664-98.
- Reid, T.M.S. 2001. 'A case study of cheese associated E. coli O157 outbreaks in Scotland' in G. Duffy, Garvey, P. and McDowell, D. A. (ed) (ed.), *Verocytotoxigenic E. coli* (Trumbull, Food and Nutrition Press Inc.: Malden, MA).
- Reitsma, Christine J., and David R. Henning. 1996. 'Survival of Enterohemorrhagic *Escherichia coli* O157:H7 During the Manufacture and Curing of Cheddar Cheese', *J Food Prot*, 59: 460-64.
- Ross, T. . 2011. 'Challenge testing of microbiological safety of raw milk cheeses: the challenge trial toolkit. ', *MAF (Ministry of Agriculture and Forestry)*, 2.
- Rosshaug, Per Sand, Ann Detmer, Hanne Ingmer, and Marianne Halberg Larsen. 2012. 'Modeling the Growth of *Listeria monocytogenes* in Soft Blue-White Cheese', *Appl Environ Microbiol*, 78: 8508-14.
- Rudolf, Melanie, and Siegfried Scherer. 2001. 'High incidence of *Listeria monocytogenes* in European red smear cheese', *International Journal of Food Microbiology*, 63: 91-98.
- Ryser, E. T., S. M. Arimi, and C. W. Donnelly. 1997. 'Effects of pH on distribution of *Listeria* ribotypes in corn, hay, and grass silage', *Appl Environ Microbiol*, 63: 3695-7.
- Ryser, Elliot T. 2012. 'Safety of Dairy Products.' in Omar A. Oyarzabal and Steffen Backert (eds.), *Microbial Food Safety: An Introduction* (Springer New York: New York, NY).
- Ryser, Elliot T., and Elmer H. Marth. 1987. 'Fate of *Listeria monocytogenes* During the Manufacture and Ripening of Camembert Cheese', *J Food Prot*, 50: 372-78.
- Ryser, Elliot T. 2007. 'Incidence and Behavior of *Listeria monocytogenes* in Cheese and Other Fermented Dairy Products.' in, *Listeria, Listeriosis, and Food Safety, Third Edition* (CRC Press).
- Saad, S. M., C. Vanzin, M. N. Oliveira, and B. D. Franco. 2001. 'Influence of lactic acid bacteria on survival of *Escherichia coli* O157:H7 in inoculated Minas cheese during storage at 8.5 degrees C', *J Food Prot*, 64: 1151-5.
- Samelis, J., A. Lianou, A. Kakouri, C. Delbes, I. Rogelj, B. Bogovic-Matijasic, and M. C. Montel. 2009. 'Changes in the microbial composition of raw milk induced by thermization treatments applied prior to traditional Greek hard cheese processing', *J Food Prot*, 72: 783-90.
- Sanaa, M., L. Coroller, and O. Cerf. 2004. 'Risk assessment of listeriosis linked to the consumption of two soft cheeses made from raw milk: Camembert of Normandy and Brie of Meaux', *Risk Anal*, 24: 389-99.
- Sauders, B. D., and D. J. D'Amico. 2016. '*Listeria monocytogenes* cross-contamination of cheese: risk throughout the food supply chain', *Epidemiol Infect*, 144: 2693-7.
- Sauders, B. D., J. Overdevest, E. Fortes, K. Windham, Y. Schukken, A. Lembo, and M. Wiedmann. 2012. 'Diversity of *Listeria* species in urban and natural environments', *Appl Environ Microbiol*, 78: 4420-33.
- SCA. 2015. 'The Specialist Cheesemakers Assured Code of Practice'. Edition 1, 2015.

- Schlesser, J. E., R. Gerdes, S. Ravishankar, K. Madsen, J. Mowbray, and A. Y. Teo. 2006. 'Survival of a five-strain cocktail of *Escherichia coli* O157:H7 during the 60-day aging period of cheddar cheese made from unpasteurized milk', *J Food Prot*, 69: 990-8.
- Schvartzman, M. S., X. Belessi, F. Butler, P. Skandamis, and K. Jordan. 2010. 'Comparison of growth limits of *Listeria monocytogenes* in milk, broth and cheese', *Journal of Applied Microbiology*, 109: 1790-99.
- Schvartzman, M. S., A. Maffre, F. Tenenhaus-Aziza, M. Sanaa, F. Butler, and K. Jordan. 2011. 'Modelling the fate of *Listeria monocytogenes* during manufacture and ripening of smeared cheese made with pasteurised or raw milk', *International Journal of Food Microbiology*, 145: S31-S38.
- Schvartzman, M. Sol, Ursula Gonzalez-Barron, Francis Butler, and Kieran Jordan. 2014a. 'Modeling the growth of *Listeria monocytogenes* on the surface of smear- or mold-ripened cheese', *Frontiers in Cellular and Infection Microbiology*, 4.
- Schvartzman, M. Sol, Ursula Gonzalez-Barron, Francis Butler, and Kieran Jordan 2014b. 'Modeling the growth of *Listeria monocytogenes* on the surface of smear- or mold-ripened cheese', *Frontiers in Cellular and Infection Microbiology*, 4: 90.
- Sharma, M., J. H. Ryu, and L. R. Beuchat. 2005. 'Inactivation of *Escherichia coli* O157:H7 in biofilm on stainless steel by treatment with an alkaline cleaner and a bacteriophage', *J Appl Microbiol*, 99: 449-59.
- Shere, J. A., K. J. Bartlett, and C. W. Kaspar. 1998. 'Longitudinal study of *Escherichia coli* O157:H7 dissemination on four dairy farms in Wisconsin', *Appl Environ Microbiol*, 64: 1390-9.
- Spano, G., E. Goffredo, L. Beneduce, D. Tarantino, A. Dupuy, and S. Massa. 2003. 'Fate of *Escherichia coli* O157:H7 during the manufacture of Mozzarella cheese', *Lett Appl Microbiol*, 36: 73-6.
- Stein, R. A., and D. E. Katz. 2017. '*Escherichia coli*, cattle and the propagation of disease', *FEMS Microbiol Lett*, 364.
- Strachan, Norval J. C., Geoffrey M. Dunn, Mary E. Locking, Thomas M. S. Reid, and Iain D. Ogden. 2006. '*Escherichia coli* O157: Burger bug or environmental pathogen?', *International Journal of Food Microbiology*, 112: 129-37.
- Strachan, Norval J. C., David R. Fenlon, and Iain D. Ogden. 2001. 'Modelling the vector pathway and infection of humans in an environmental outbreak of *Escherichia coli* O157', *FEMS Microbiol Lett*, 203: 69-73.
- Synge, B. A., M. E. Chase-Topping, G. F. Hopkins, I. J. McKendrick, F. Thomson-Carter, D. Gray, S. M. Rusbridge, F. I. Munro, G. Foster, and G. J. Gunn. 2003. 'Factors influencing the shedding of verocytotoxin-producing *Escherichia coli* O157 by beef suckler cows', *Epidemiol Infect*, 130: 301-12.
- Tham, W. A., L. J. Hajdu, and M. L. Danielsson-Tham. 1990. 'Bacteriological quality of on-farm manufactured goat cheese', *Epidemiol Infect*, 104: 87-100.
- Thiran, E., P. A. Di Ciccio, H. U. Graber, E. Zanardi, A. Ianieri, and J. Hummerjohann. 2017. 'Biofilm formation of *Staphylococcus aureus* dairy isolates representing different genotypes', *Journal of Dairy Science*.
- Threlfall, E. J., J. A. Frost, L. R. Ward, and B. Rowe. 1996. 'Increasing spectrum of resistance in multiresistant *Salmonella* Typhimurium', *The Lancet*, 347: 1053-54.
- Toft, Nils, Giles T. Innocent, Iain J. McKendrick, Helen E. Ternent, Dominic J. Mellor, George J. Gunn, Barti Synge, and Stuart W. J. Reid. 2005. 'Spatial distribution of *Escherichia coli* O157-positive farms in Scotland', *Preventive Veterinary Medicine*, 71: 45-56.
- Tondo, E. C., M. C. Guimaraes, J. A. Henriques, and M. A. Ayub. 2000. 'Assessing and analysing contamination of a dairy products processing plant by *Staphylococcus aureus* using antibiotic resistance and PFGE', *Can J Microbiol*, 46: 1108-14.

- Trmcic, A., K. Chauhan, D. J. Kent, R. D. Ralyea, N. H. Martin, K. J. Boor, and M. Wiedmann. 2016. 'Coliform detection in cheese is associated with specific cheese characteristics, but no association was found with pathogen detection', *J Dairy Sci*, 99: 6105-20.
- Trmčić, A., R. Ralyea, L. Meunier-Goddik, C. Donnelly, K. Glass, D. D'Amico, E. Meredith, M. Kehler, N. Tranchina, C. McCue, and M. Wiedmann. 2017. 'Consensus categorization of cheese based on water activity and pH—A rational approach to systemizing cheese diversity', *Journal of Dairy Science*, 100: 841-47.
- Tuan Zainazor, C., M. S. Hidayah, L. C. Chai, R. Tunung, F. M. Ghazali, and R. Son. 2010. 'The scenario of norovirus contamination in food and food handlers', *J Microbiol Biotechnol*, 20: 229-37.
- Venegas-Vargas, Cristina, Scott Henderson, Akanksha Khare, Rebekah E. Mosci, Jonathan D. Lehnert, Pallavi Singh, Lindsey M. Ouellette, Bo Norby, Julie A. Funk, Steven Rust, Paul C. Bartlett, Daniel Grooms, and Shannon D. Manning. 2016. 'Factors Associated with Shiga Toxin-Producing *Escherichia coli* Shedding by Dairy and Beef Cattle', *Appl Environ Microbiol*, 82: 5049-56.
- Verdier-Metz, Isabelle, Geneviève Gagne, Stéphanie Bornes, Françoise Monsallier, Philippe Veisseire, Céline Delbès-Paus, and Marie-Christine Montel. 2012. 'Cow Teat Skin, a Potential Source of Diverse Microbial Populations for Cheese Production', *Appl Environ Microbiol*, 78: 326-33.
- Vernozy-Rozand, C., C. Mazuy-Cruchaudet, C. Bavai, M. P. Montet, V. Bonin, A. Dernburg, and Y. Richard. 2005a. 'Growth and survival of *Escherichia coli* O157:H7 during the manufacture and ripening of raw goat milk lactic cheeses', *Int J Food Microbiol*, 105: 83-8.
- Vernozy-Rozand, C., M. P. Montet, M. Berardin, C. Bavai, and L. Beutin. 2005b. 'Isolation and characterization of Shiga toxin-producing *Escherichia coli* strains from raw milk cheeses in France', *Letters in Applied Microbiology*, 41: 235-41.
- Viçosa, Gabriela Nogueira, Paula Mendonça Moraes, Anderson Keizo Yamazi, and Luís Augusto Nero. 2010. 'Enumeration of coagulase and thermonuclease-positive *Staphylococcus* spp. in raw milk and fresh soft cheese: An evaluation of Baird-Parker agar, Rabbit Plasma Fibrinogen agar and the Petrifilm™ Staph Express count system', *Food Microbiology*, 27: 447-52.
- Villar, R. G., M. D. Macek, S. Simons, and et al. 1999. 'Investigation of multidrug-resistant *salmonella* serotype typhimurium dt104 infections linked to raw-milk cheese in washington state', *JAMA*, 281: 1811-16.
- Vogeleer, Philippe, Yannick D. N. Tremblay, Grégory Jubelin, Mario Jacques, and Josée Harel. 2016. 'Biofilm-Forming Abilities of Shiga Toxin-Producing *Escherichia coli* Isolates Associated with Human Infections', *Appl Environ Microbiol*, 82: 1448-58.
- Vogeleer, Philippe, Yannick D. N. Tremblay, Akier A. Mafu, Mario Jacques, and Josée Harel. 2014. 'Life on the outside: role of biofilms in environmental persistence of Shiga-toxin producing *Escherichia coli*', *Frontiers in Microbiology*, 5: 317.
- Voysey, P.A. , R.A. Green, C.J. Baylis, K.J. Bridgewater, and Anslow P. 2012. 'Mycobacterium bovis and Verocytotoxin-producing *Escherichia coli* (VTEC) in UK made raw milk cheese. '.
- Waldman, Kurt B., and John M. Kerr. 2018. 'Does safety information influence consumers' preferences for controversial food products?', *Food Quality and Preference*, 64: 56-65.
- Wells, S. J., P. J. Fedorka-Cray, D. A. Dargatz, K. Ferris, and A. Green. 2001. 'Fecal shedding of *Salmonella* spp. by dairy cows on farm and at cull cow markets', *J Food Prot*, 64: 3-11.
- White, Charles W., and Edward W. Custer. 1976. 'Survival of *Salmonella* in Cheddar Cheese', *Journal of Milk and Food Technology*, 39: 328-31.
- WHO. 2004. 'Risk assessment of L.m. in ready to eat foods'.

- WHO. 2016. "WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007–2015." ISBN 978 92 4 156516 5
- Williams, Alan G., and Susan E. Withers. 2010. 'Microbiological characterisation of artisanal farmhouse cheeses manufactured in Scotland', *International Journal of Dairy Technology*, 63: 356-69.
- Wolfe, Benjamin E, Julie E Button, Marcela Santarelli, and Rachel J Dutton. 2014. 'Cheese Rind Communities Provide Tractable Systems for In Situ and In Vitro Studies of Microbial Diversity', *Cell*, 158: 422-33.
- Yoon, Yohan, Soomin Lee, and Kyoung-Hee Choi. 2016. 'Microbial benefits and risks of raw milk cheese', *Food Control*, 63: 201-15.
- Zárate, V., F. Belda, C. Pérez, and E. Cardell. 1997. 'Changes in the microbial flora of Tenerife goats' milk cheese during ripening', *International Dairy Journal*, 7: 635-41.

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