## European Commission Co-ordinated Programme for the Official Control of Foodstuffs for 2004:

## Microbiological Examination of Cheeses made from Raw or Thermised Milk from Production Establishments and Retail Premises in the United

#### Kingdom

CL Little<sup>1\*</sup>, JR Rhoades<sup>1</sup>, SK Sagoo<sup>1</sup>, M Greenwood<sup>2</sup> V Mithani<sup>1</sup>, K Grant<sup>1</sup>, J MCLauchlin<sup>1</sup> and the Food, Water and Environmental Surveillance Network<sup>†</sup>. <sup>1</sup> Department of Gastrointestinal Infections, Centre for Infections, Health Protection Agency, 61 Colindale Avenue, London NW9 5EQ. <sup>2</sup> Health Protection Agency Wessex Environmental Microbiological Services, Southampton General Hospital, Southampton, UK

\*, Corresponding author, †, FWES Network comprises Laboratories listed in Annex 1

#### On behalf of the Food Standards Agency, Local Authorities Coordinators of Regulatory Services and the Health Protection Agency

#### Summary

As part of the European Commission co-ordinated programme for the official control of foodstuffs for 2004, a study of fresh, ripened and semi-hard cheeses made from raw or thermised milk from retail and production premises was undertaken in the UK to determine the microbiological quality of these According to microbiological criteria in EC Recommendation products. 2004/24/EC, 98% of the 1842 samples from retail and batches from production were of satisfactory/borderline microbiological quality. Likewise, in the following study in 2005 of pasteurised milk cheeses, 98% were also found also to be of satisfactory/borderline microbiological quality according to Recommendation 2005/175/EC. Two percent of samples in the present study were of unsatisfactory quality due to high levels of Staphylococcus aureus  $(\geq 10^4 \text{ cfu/g})$ , Escherichia coli  $(\geq 10^5 \text{ cfu/g})$ , Listeria monocytogenes  $(\geq 10^2 \text{ cfu/g})$ or presence of Campylobacter spp (1 sample). Salmonella spp. was not detected in any samples. Raw or thermised milk cheeses were more likely to be of unsatisfactory microbiological quality when they were: unripened cheese varieties; sampled from retail premises without a hazard analysis system in place, and rated as having little or no confidence in management and control systems; stored or displayed above 8°C. Evidence from this study also indicates that labelling of cheeses with clear information on whether the cheese was prepared from raw milk requires improvement.

#### Introduction

Cheese making is a major industry worldwide, and much is still practised on a relatively small scale which accounts for the rich diversity of cheeses available<sup>1</sup>. Classification of cheeses is made difficult by this diversity but the most widely accepted approach is one based on moisture content, with further subdivision depending on milk type and the role of microorganisms in cheese ripening. The attribute of 'softness' or 'hardness' is therefore directly related to the moisture content of the cheese, higher moisture cheeses being softer than low moisture cheeses. Cheese consists primarily of milk fat and coagulated proteins and preservation is primarily achieved by controlling two physico-chemical parameters: pH and water activity. Reduction in pH is achieved by fermentation of lactose by starter culture organisms (lactic acid bacteria) and/or addition of acid. Water activity is reduced by pressing of the whey from the curd, and by salting and drying<sup>1</sup>. Other intrinsic parameters that may affect the growth and survival of microorganisms in cheeses are redox potential<sup>2</sup> and the presence of anti-microbial compounds produced by starter and non-starter organisms<sup>3,4,5</sup>. These properties of cheese, together with the length of maturation of the finished product and the fact that they are normally stored at a controlled temperature, constitute a 'hurdle' system of preservation that act as control steps to inhibit the growth of pathogenic bacteria.

Whilst the efficient pasteurisation of milk should eliminate the risk from viable pathogenic organisms, cheese can be made safely with raw milk. Many cheesemakers use raw milk or add raw milk to the cheese milk, considering it essential for good flavour, primarily due to greater proteolysis and lipolysis by the raw milk microflora in the cheese. Some cheeses are also made with thermised milk that has been given a sub-pasteurisation temperature (57°C to 68°C for at least 15 seconds) designed to eliminate spoilage bacteria. The manufacture of cheese is well regulated in the UK, production at the time of this study was controlled by the Dairy Products (Hygiene) Regulations 1995<sup>6</sup>. From 1 January 2006 these Regulations were superseded by the new EU food hygiene regulations that apply directly to Member States<sup>7,8</sup>. For unpasteurised milk cheese, milk production is the first critical control point

(CCP) in the cheesemaker's Hazard Analysis and Critical Control Point (HACCP) plan. *Staphylococcus aureus* is the commonest cause of mastitis in diary cows, and faecal contamination of milk during milking also poses a risk of introducing pathogens. The microbiological quality of cheese is also influenced by equipment and environment hygiene during manufacture, packaging and handling<sup>9</sup>. The Specialist Cheesemakers Association has produced a Code of Best Practice in the manufacture of cheese for UK producers to help minimize microbial food safety hazards<sup>10</sup>, and the Food Standards Agency (FSA) and ADAS also set up the Specialist Cheesemakers Initiative to assist cheesemakers in implementing HACCP principles<sup>11</sup>.

Although cheeses are currently considered to be some of the safest foods consumed, pathogenic bacteria that can be transmitted by dairy products, including cheese, are important to the dairy industry. Historically there have been outbreaks of infection associated with the consumption of cheese, and the predominant organisms responsible have included *Salmonella* spp., *Listeria monocytogenes,* verocytotoxin producing *Escherichia coli* (VTEC), and *Staphylococcus aureus*<sup>12-14</sup>. Detailed investigations have demonstrated that the source of contamination was raw milk, inadequately pasteurised milk, or post-pasteurisation contamination with organisms originally derived from raw milk or from manufacturing environments. People at high risk from listeriosis, including pregnant women, are advised in the UK not to consume soft mould-ripened cheeses or blue cheeses<sup>15</sup>. Mandatory labelling of cheeses made from raw milk also has been introduced in Europe so that the consumer can make an informed choice of purchase<sup>8</sup>.

All member states are required by the EC to carry out a co-ordinated sampling programme for the official control of foodstuffs. The Local Authorities Co-ordinators of Regulatory Services (LACORS) and the Health Protection Agency (HPA) Co-ordinated Food Liaison Group programme undertook two such studies in 2004 and 2005 on the microbiological quality of cheeses from retail and production premises in the UK<sup>16,17</sup>, one on cheeses made using raw or thermised milk and the other on those made using pasteurised milk.

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Reported here are the results of the first of these studies on cheeses made from raw or thermised milk from retail and production premises.

### **Materials and Methods**

#### Sample collection

Unripened (fresh) or ripened soft and semi-hard cheeses made from raw or thermised milk were collected from retail and production premises and examined by 33 laboratories (Health Protection Agency (HPA), HPA Collaborating Laboratories, National Public Health Service (NPHS)-Wales and Public Analysts) in the UK between 1 September and 31 October 2004 according to a standardised protocol. Cheeses made from cows', ewes', goats', and buffalo milk were included. Five sample units were collected from each batch at production premises according to class attribute sampling plans as provided in Commission Recommendation 2004/24/EEC, whereas single samples were collected from retail premises<sup>16</sup>. Samples (5 x 100g from production, 100g from retail) were collected and transported to laboratories by staff from 264 local Environmental Health Departments, involving 271 Local Authority Food Liaison Groups (Annex 1), in accordance with the FSA Food Law Code of Practice<sup>18</sup> and LACORS guidance on microbiological food sampling<sup>19</sup>.

Information on samples and premises was obtained by observation and enquiry and recorded on a standard proforma. Additional information collected included the type of cheese, country of origin, packing details, display/storage temperature, existence of a hazard analysis system and the level of food hygiene training received by the manager. Food hygiene inspections are carried out in a way that focuses enforcement authority resources on premises presenting most risk to consumers. To do this, food hygiene inspections are carried out in accordance with FSA Food Law Code of Practice<sup>18</sup> which specifies that, amongst other factors, the number of consumers at risk and confidence in management control systems (including the application of HACCP based systems) should be assessed to produce a risk rating of the premises. The risk rating determines the frequency of

inspection and at the time of this study ranged from Category A (highest risk, inspected every 6 months) to F (lowest risk, inspected every 5 years).

#### Sample examination

Salmonella spp., L. monocytogenes and other Listeria spp., S. aureus and E. coli were enumerated or their presence sought in accordance with HPA Standard Microbiological Methods<sup>20-23</sup>. Campylobacter spp. were detected by enrichment in Bolton Selective Enrichment Broth with incubation at 37°C for 4 hours, followed by further incubation at 41.5°C and subculture to Campylobacter selective agar (CCDA) after 44±2 h. Inoculated plates were incubated at 41.5°C for 48 h, and colonies identified as described in HPA Standard Microbiological Method F21<sup>24</sup>. Isolates of *Campylobacter* spp. were sent to the Laboratory of Enteric Pathogens (LEP), Health Protection Agency Centre for Infections (HPA CfI), for typing and further characterisation. All isolates of L. monocytogenes, and other species of Listeria at high levels ( $\geq 100 \text{ cfu/g}$ ) were sent to the Food Safety Microbiology Laboratory (FSML), HPA Cfl for further characterisation. For *L. monocytogenes* this included sero-typing and amplified fragment length polymorphism (AFLP) as described previously<sup>25,26</sup>. Isolates of S. aureus at  $\geq 10^4$  cfu/g were also sent to FSML to determine the enterotoxin gene fragments by polymerase chain reaction (PCR)<sup>27</sup>.

The microbiological status of production batches of cheese were assessed using the class attributes plans stipulated in Recommendation 2004/24/EC<sup>16</sup> (Table 1). The microbiological status of single retail samples of cheese were also assessed using the criteria in Recommendation 2004/24/EC<sup>16</sup> (Table 2)

Table 1. Microbiological criteria	for	batch	samples	from	production	premises
(Recommendation 2004/24/EC <sup>16</sup> )						

Microorganism	Microbiological criteria				
Escherichia coli	n= 5 c=2 m= 10 <sup>4</sup> cfu/g M=10 <sup>5</sup> cfu/g				
Staphylococcus aureus	n= 5 c=2 m= $10^3$ cfu/g M= $10^4$ cfu/g				
Listeria monocytogenes	n=5 c=0 Absent in 25g				
Campylobacter spp.	n=5 c=0 Absent in 25g				
Salmonella spp.	n=5 c=0 Absent in 25g				
Where parameters n, m, M and c are defined as follows:					
n = number of units comprising	the sample				

n = number of units comprising the sample

m = limit below which all results are considered satisfactory

M = acceptability limit beyond which the results are considered unsatisfactory

 $\mathsf{c}$  = number of sampling units giving bacterial counts of between  $\mathsf{m}$  and  $\mathsf{M}$ 

For *E. coli* and *S. aureus* (guideline criterion) the status of a batch is:

- Satisfactory where all the values are less than m
- Borderline acceptability where the maximum of c values are between m and M
- Unsatisfactory if one or more values is/are above M or more than c values between m and M

For *L. monocytogenes* the status of a batch is:

- Satisfactory if not detected in 25g
- Borderline acceptability if detected and <100 cfu/g
- Unsatisfactory if detected and ≥100 cfu/g

For Salmonella spp. and Campylobacter spp. the status of a batch is:

- Satisfactory where all the values are not detected in 25g
- Unsatisfactory where one or more values are detected in 25g

Table	2:	Microbiological	criteria	for	single	samples	from	retail	premises
(Recor	nme	endation 2004/24/	′EC <sup>16</sup> )		_				

Microorganism	Satisfactory	Borderline	Unsatisfactory
Escherichia coli	<10 <sup>4*</sup>	10 <sup>4</sup> - <10 <sup>5</sup>	≥10 <sup>5</sup>
Staphylococcus aureus	<10 <sup>3</sup>	10 <sup>3</sup> - <10 <sup>4</sup>	≥10 <sup>4</sup>
Listeria monocytogenes	ND	Detected - <10 <sup>2</sup>	≥10 <sup>2</sup>
Campylobacter spp.	ND	-	Detected
Salmonella spp.	ND	-	Detected

\*, cfu/g

#### **Statistical Analysis**

Descriptive and statistical analysis of the data was undertaken using Microsoft Excel and Epi Info version 6.04d. Relative proportions were compared using chi-squared ( $\chi^2$ ) and fisher's exact test. A probability value of less than 5% was deemed to be significant.

#### **Results**

#### Microbiological status of cheeses made from raw or thermised milk

#### Production Establishments

Twenty-one production establishments were visited. Eight unripened (fresh) soft cheese, eight ripened soft cheese, and seven semi-hard cheese batches were tested; five sample units were collected per batch; therefore in total 115 sample units were examined.

Applying the criteria in Recommendation 2004/24/EC<sup>16</sup>, 78% (18/23) of batches were of satisfactory microbiological quality, 18% (4) were of unsatisfactory quality due to high levels of *S. aureus* (3) or *E. coli* (1), and one (4%) batch was of unsatisfactory quality due to the presence of *L. monocytogenes* in excess of 100 cfu/g (210 cfu/g) (Table 3).

Table 3. Microbiological quality of cheeses made from raw or thermised milk
according to Recommendation 2004/24/EC <sup>16</sup> from production premises in the
UK

Microorganism	Product	Number of		Analysis results	5
	Identification	samples	Satisfactory	Borderline	Unsatisfactory
Salmonella spp.	Unripened soft (Fresh)	8	8	0	0
n=5 c=0 absent in 25g	Ripened Soft	8	8	0	0
Ū Ū	Semi-Hard	7	7	0	0
	Unripened soft (Fresh)	8	8	0	0
Campylobacter spp.	Ripened Soft	8	8	0	0
n=5 c=0 absent in 25g	Semi-Hard	7	7	0	0
Staphylococcus aureus	Unripened soft (Fresh)	8	6	0	2
n=5 c=2 m=1000 cfu/g M=10,000	Ripened Soft	8	7	0	1
cfu/g	Semi-Hard	7	7	0	0
Escherichia coli	Unripened soft (Fresh)	8	8	0	0
n=5 c=1 m=10,000 cfu/g	Ripened Soft	8	7	0	1
M=100,000 cfu/g	Semi-Hard	7	7	0	0
			*ND	<100cfu/g	≥100cfu/g
	Unripened soft (Fresh)	8	8	0	0
Listeria monocytogenes	Ripened Soft	8	8	0	0
n=5 c=0 absent in 25g	Semi-Hard	7	6	0	1

\*ND, Not detected in 25g

Two of the three batches that had high *S. aureus* counts were unripened soft raw goats' milk cheese products from different batches produced by the same on farm dairy products producer. One batch contained *S. aureus* ranging from 1.9 x 10<sup>4</sup> to 3.6 x 10<sup>4</sup> cfu/g from the five sample units; another batch contained *S. aureus* at 1.3 x 10<sup>4</sup> cfu/g in one sample unit, 3.5 x 10<sup>3</sup> to 5.8 x 10<sup>3</sup> cfu/g from a further three sample units, while the remaining sample unit contained <20 cfu/g. *S. aureus* isolates from all these sample units contained the staphylococcal enterotoxin (SE) C gene fragment. The other batch was a ripened soft blue raw cows' milk cheese containing *S. aureus* in two of the sample units at levels of 5.2 x 10<sup>4</sup> and 5.5 x 10<sup>5</sup> cfu/g (SE gene fragments were not detected), while the other three sample units had *S. aureus* present at levels < 20 cfu/g. One batch of soft ripened cheese made from raw ewes' milk had high *E. coli* levels ranging from 2.0 x 10<sup>4</sup> to 2.4 x 10<sup>5</sup> cfu/g in four sample units, and 75 cfu/g in the remaining sample unit. A batch of semi-hard raw cows' milk cheese had *L. monocytogenes* (serotype/AFLP: 1/2a/VII) present in excess of  $10^2$  cfu/g in one of the five samples units (210 cfu/g), while the organism was not detected in the remaining four sample units.

As there were only 23 batches of cheese sampled from production sites, statistical analysis of the results and a comprehensive investigation of the influence of different parameters on cheese quality were not possible. Production premises and product information collected for these 23 batches of cheese are presented in Tables 4 and 5.

Table 4. Microbiological quality of raw or thermised milk cheeses according to Recommendation 2004/24/EC<sup>16</sup> in relation to production product details

<b>Product Details</b>	No. Ba	atches	No. Batches of Unsatisfactory
	(n=23)	%	Quality (n=5)
Cheese type			
Unripened soft	8	35	2
Ripened soft	8	35	2
Semi-hard	7	30	1
Milk species			
Cows' milk	10	44	1
Goats' milk	8	35	3
Ewes milk	4	17	1
Other (e.g. buffalo)	1	4	-
Not recorded		-	-
Milk type			
Raw milk	17	74	5
Thermised milk	5	22	-
Not recorded	1	4	-
FSA/ADAS Specialist Cheesema	kers Initiative		
Participated	17	74	4
Not participated	5	22	1
Not Known	1	1	-
Labelled as organic			
Yes	4	17	-
No	16	70	5
Not recorded	3	13	
Display/Storage Temperature			
≤8°C	16	70	3
>8°C	4	17	1
Not recorded	3	13	1

## Table 5. Microbiological quality of raw or thermised milk cheeses according to Recommendation 2004/24/EC<sup>16</sup> in relation to production premises details

Premises Details		No. Ba	atches	No. Batches of	
		(n =23)	(%)	Unsatisfactory Quality (n=5)	
Premises T	уре				
	iry producer	14	61	3	
	icts producer (non-farm)	6	26	2	
Not recorde		3	13	-	
Inspection F	Rating Category				
Category	Minimum Frequency of Inspection				
A	At least every 6 months	9	39	3	
В	At least every year	10	43	2	
С	At least every 18 months	3	13	-	
	Not recorded	1	4	_	
Consumer a	at Risk Score				
0 (Very few	)	-	-	-	
5 (Few)		4	17	1	
10 (Interme	diate)	10	43	2	
15 (Substar		8	35	2	
Not recorde	,	1	4	-	
Confidence	in Management				
0 (High)	-	4	17	-	
5 (Moderate	e)	6	26	1	
10 (Some)		11	48	3	
20 (Little)		1	4	1	
30 (None)		-	-	-	
Not recorde	ed	1	4	-	
	alysis Systems				
	d documented	21	91	5	
Not in place		-	-	-	
Not recorde		2	9	-	
Manageme	nt Food Hygiene Training				
	aining and attended				
Basic 6 hou		10	48	3	
Intermediate	e course	2	9	2	
Advanced c		3	13	-	
	nised course	4	17	-	
No training	,	-	-	-	
Not recorde	h	3	13	_	

#### **Retail premises**

A total of 1819 samples of cheeses were tested, of which 62 (3.4%) were unripened (fresh) soft cheese, 806 (44.4%) were ripened soft cheese, and 951 (52.2%) were semi-hard cheese samples.

#### Microbiological quality in relation to Recommendation 2004/24/EC

Applying the criteria in Recommendation 2004/24/EC<sup>16</sup>, 96% (1742/1819) of samples were of satisfactory microbiological quality, 2% (40) were of borderline quality, and a further 2% (32) were of unsatisfactory quality due to high levels of *S. aureus* (ranging from 1.6x  $10^5$  to  $>10^7$ cfu/g) and/or *E. coli* (ranging from 1.1 x  $10^5$  to 4.6 x  $10^6$  cfu/g), the presence of *Campylobacter* 

*jejuni* (one sample), and *L. monocytogenes* present at over  $10^2$  cfu/g (220 cfu/g) (one sample) (Table 6, Fig. 1). *Salmonella* spp. was not detected in any samples examined. Overall contamination of *Listeria* spp. in cheeses was 3.1% (56). *L. innocua* was also present in one sample at over  $10^2$  cfu/g (8.3 x  $10^3$  cfu/g).

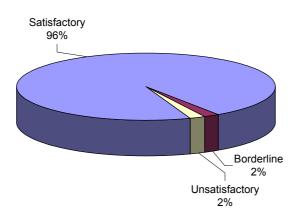
Table 6. Microbiological quality of cheeses made from raw or thermised milk
according to Recommendation 2004/24/EC <sup>16</sup> from retail premises in the UK

Microorgonicm	Product	Number of		Analysis resu	lts
Microorganism	Identification	samples	Satisfactory	Borderline	Unsatisfactory
Salmonella spp.	Unripened soft (Fresh)	62	62	0	0
n=5 c=0 absent in 25g	Ripened Soft	806	806	0	0
0	Semi-Hard	951	951	0	0
	Unripened soft (Fresh)	62	62	0	0
Campylobacter spp	Ripened Soft	806	806	0	0
n=5 c=0 absent in 25g	Semi-Hard	951	950	0	1
Staphylococcus aureus	Unripened soft (Fresh)	62	59	2	1
n=5 c=2 m=1000 cfu/g	Ripened Soft	806	787	9	10 <sup>†</sup>
M=10,000 cfu/g	Semi-Hard	951	947	2	2
Escherichia coli	Unripened soft (Fresh)	62	60	0	2
n=5 c=1 m=10,000 cfu/g	Ripened Soft	806	773	17	16 <sup>†</sup>
M=100,000 cfu/g	Semi-Hard	951	935	9	7
			*ND	<100 cfu/g	≥100 cfu/g
	Unripened soft (Fresh)	62	61	1	0
Listeria monocytogenes	Ripened Soft	806	798	8	0
n=5 c=0 absent in 25g	Semi-Hard	951	943	7	1

\*ND, Not detected in 25g

†Two samples had unsatisfactory levels of both S. aureus and E. coli

# Figure 1. Microbiological quality of retail soft and semi-hard cheeses made from raw or thermised milk from retail premises using criteria in Recommendation 2004/24/EC<sup>16</sup> (n=1819)



Ten of the 13 cheeses containing S. *aureus* at  $10^4$  cfu/g or more were ripened soft cheeses, one was an unripened soft cheese, and two were semi-hard cheeses. Six of these cheeses were pre-packed, six were cut to order, and for one sample this information was not recorded. The sample containing *C. jejuni* (HS 8) was an Irish goats' fresh soft milk cheese sampled from a supermarket delicatessen. The sample that had *L. monocytogenes* (serotype/AFLP: 1/2a/IX) present in excess of  $10^2$  cfu/g was a cut-to-order semi-hard smear-ripened Scottish cheese sampled from a specialist cheese shop.

#### Genes for Staphylococcal Enterotoxin (SE) Production

*S. aureus* isolates from four of the 13 cheese samples where the bacteria was present at  $\geq 10^4$  cfu/g all had genes for staphylococcal enterotoxin (SE) (Table 7). Five different SE gene fragments were amplified from these isolates, three of which were obtained from ripened soft cheese samples produced from raw milk.

Milk type	Cheese type	No. Samples	Staphylococcal enterotoxin gene fragments detected
Raw	Ripened soft	1	G, I
Raw	Semi hard	1	G, I
Raw	Ripened soft	1	D, J
Raw	Ripened soft	1	G, H, I

Table 7. *S. aureus* isolates containing genes for staphylococcal enterotoxin recovered from retail raw milk cheeses

#### L. monocytogenes isolates present in raw or thermised milk cheeses

*L. monocytogenes* was detected in 17 (1%) of the 1819 samples. Seven of the nine referred isolates were serotype 1/2a (Table 8). Four different *L. monocytogenes* subtypes were obtained from isolates recovered from the nine samples (Table 8).

Milk type	Cheese type	No. samples	L. monocytogenes subtype (Serotype/AFLP*)
Raw	Ripened soft	3	1/2a/VII
Raw	Semi hard	2	1/2a/IX
Not known	Ripened soft	1	
Raw	Semi hard	1	1/2a/XI
Raw	Semi hard	2	4b/V
	Ripened soft		

Table 8. Subtypes of *L. monocytogenes* isolated from retail raw or thermised milk cheeses

\*, Amplified fragment length polymorphism;

#### Product information in relation to microbiological quality

Analysis of data on retail cheese samples and product information was carried out using the criteria within Recommendation 2004/24/EC<sup>16</sup> (Table 2).

#### Product details

Amongst the 1819 cheeses sampled, 52% were semi-hard cheeses (e.g. Emmental, Roquefort, Port Salut), 45% were ripened soft cheeses (e.g. Brie, Camembert), and 3% were unripened soft cheeses (e.g. cream cheese, Ricotta) (Table 9). More samples of unripened soft cheese (4.8%) were of unsatisfactory quality compared with ripened soft (2.9%) and semi-hard cheeses (1.2%) (Table 9). This finding was only significantly different when comparing unripened soft cheeses to semi-hard cheeses (p=0.0490).

Fifty-nine percent of samples collected were made using cows' milk, 19% from goats' milk, 8% from ewes' milk, and 1% were made from milk from other animal species (e.g. buffalo). This information was not recorded for 13% (Table 9). Four percent of goats' milk cheese samples were of unsatisfactory microbiological quality which was a higher proportion compared to cheeses made from milk from other animals (cows' (2.7%), ewes' (0.3%)) (Table 9). This finding was only significant when comparing goats' milk cheese with ewes' milk cheese (p=0.0044).

Of the 1819 samples sampled, 79% were made using raw milk, 6% from thermised milk: this information was not recorded for 15% (Table 9). The proportion of raw milk cheeses of unsatisfactory quality was higher (2.4%)

when compared to thermised milk cheeses (0.9%), although this finding was not statistically significant (p=0.5122).

<b>Product Details</b>	No. Samples		No. Samples of Unsatisfactory
	n =1819	%	Quality (%)
Cheese type			
Unripened soft	62	3	3 (4.8%)
Ripened soft	806	45	23 (2.9%)
Semi-hard	951	52	11 (1.2%)
Milk species			
Cows' milk	1071	59	29 (2.7%)
Goats' milk	156	8	6 (3.8%)
Ewes milk	346	19	1 (0.3%)
Other (e.g. buffalo)	11	1	0
Not recorded	235	13	1 (0.4%)
Milk Thermal processing			
Raw milk	1428	79	34 (2.4%)
Thermised milk	116	6	1 (0.9%)
Not recorded	275	15	2 (0.7%)
Labelled as organic			
Yes	70	4	2 (2.9%)
No	1662	91	34 (2.1%)
Not recorded	87	5	1 (1.2%)
Packaging			
Pre-packed	964	53	23 (2.4%)
Cut to order	762	42	13 (1.7%)
Not recorded	93	5	1 (1.1%)
Pack size (n=964)			
<50 g	14	1	0
50–<100 g	188	20	2 (1.1%)
100–<200 g	543	58	11 (2.0%)
200–<300 g	162	17	8 (4.9%)
300–<400 g	16	2	0
≥400 g	19	2	2 (10.5%)
Not recorded	22		0
Display/ Storage Temperature			
≤8°C	1653	90	31 (1.9%)
>8°C	85	5	6 (7.1%)
Not recorded	81	5	0

## Table 9. Microbiological quality of retail raw or thermised milk cheeses in relation to product details

Ninety-one percent of the 1819 cheese samples were not labelled as organic products (Table 9). The proportion of cheeses labelled or not labelled as organic products of unsatisfactory quality was similar, 2.1% and 2.9% respectively. However, it should be noted that the proportion of samples labelled as organic examined was very small and that no statistical conclusions should be drawn from these results.

Fifty-two percent of the 1819 cheeses collected were pre-packed. Of the samples that were pre-packed, over half (58%) had a pack size of between 100 to 200g (Table 9). There was no significant difference in the proportion of unsatisfactory cheese samples between those that were pre-packed (2.4%) or cut to order (1.7%) (p=0.3973).

The majority (90%) of cheese samples were stored or displayed at or below  $8^{\circ}$ C (Table 9). A higher proportion of cheese samples (7.1%) that were stored above  $8^{\circ}$ C were of unsatisfactory microbiological quality compared to those stored below  $8^{\circ}$ C (1.9%) (p=0.0078).

Overall 75% of the cheeses sampled were labelled as having being produced from unpasteurised milk, 18% were not and for 6% of samples, this information was not recorded. A higher proportion of raw milk cheeses had this labelling (82%) compared to cheeses made from thermised milk (46%).

#### Country of origin

Cheeses collected from retail premises were produced in 14 countries (Table 10), with 47.2% produced in France, 19.3% in the UK, 7.0% in Switzerland, 6.2% in Denmark, and 5.8% in Italy. A higher proportion of cheeses produced in the Republic of Ireland were of unsatisfactory quality (6.7%) compared to those produced in the UK (3.4%), France (2.4%), Switzerland (1.6%) and elsewhere. However, it should be noted that the proportion of samples produced in the Republic of Ireland is comparatively small compared to those produced in, for example the UK and France. Therefore no statistical conclusions should be drawn from these results.

Country of origin	No. Samples		No. Samples of Unsatisfactory
	n=1819	%	Quality (%)
UK	350	19.3	12 (3.4%)
Other EU	1,138	62.6	23 (2.0%)
Austria	7	0.4	-
Cyprus	2	0.1	-
Denmark	112	6.2	-
France	859	47.2	21 (2.4%)
Germany	6	0.3	-
Greece	3	0.2	-
Italy	105	5.8	-
Netherlands	7	0.4	-
Republic of Ireland	30	1.6	2 (6.7%)
Spain	7	0.4	-
Non- EU	131	7.2	2 (1.5%)
Canada	2	0.1	-
Norway	1	0.1	-
Switzerland	128	7.0	2 (1.6%)
Not Known	200	11.0	<u>-</u>

Table 10. Microbiological quality of retail raw or thermised milk cheeses in relation to country of origin

#### Premises details in relation to microbiological quality

#### Type of Premises

Fifty-three percent of the 1819 cheeses sampled were collected from supermarkets and supermarket delicatessens. The remaining 47% were collected from delicatessens (23%), specialist cheese shops (8%), farm shops (5%), markets (4%; including farmers' markets), and for 6% of samples, this information was not recorded (Table 11). The proportion of cheese samples from farm shops and markets (8.4%) of unsatisfactory microbiological quality was significantly higher when compared to those collected from other premises (1.0% - 2.7%) (Table 11) (p=0.0002).

#### Food Hygiene Inspections

Fifty eight percent of samples were collected from premises categorised as inspection rating Category C (inspected at least every 18 months) (Table 11). More cheese samples of unsatisfactory microbiological quality were collected from premises with an inspection rating category B (3.1%) or C (2.2%) than premises with ratings of D (1.0%).

## Table 11. Microbiological quality of retail raw or thermised milk cheeses in relation to retail premises details

Retail premises details	No. Samples		No. Samples of
	n =1819	(%)	Unsatisfactory Quality (%)
Premises Type			
Supermarket (pre-packed)	735	(40)	7 (1.0%)
Supermarket (deli.)	237	(13)	5 (2.1%)
Delicatessen	427	(23)	9 (2.1%)
Specialist cheese shop	147	(8)	4 (2.7%)
Farm shop	98	(5)	7 (7.1%)
Farmers' market	9	(<1)	2 (22.2%)
Other market	60	(3)	1 (1.7%)
Other (e.g. butchers, health food shop, grocers	106	(6)	2 (1.9%)
Inspection Rating Category			
Category Minimum Frequency of Inspection			
A At least every 6 months	30	(2)	0
B At least every year	224	(12)	7 (3.1%)
C At least every 18 months	1053	(58)	23 (2.2%)
D At least every 2 years	209	(11)	2 (1.0%)
E At least every 3 years	55	(3)	0
F At least every 5 years	32	(2)	1 (3.1%)
Not recorded	216	(2)	4 (1.9%)
Consumer at Risk Score			
0 (Very few)	12	(1)	3 (25.0%)
5 (Few)	930	(51)	23 (2.5%)
10 (Intermediate)	590	(32)	6 (1.0%)
15 (Substantial)	53	(3)	1 (1.9%)
Not recorded	234	(13)	4 (1.7%)
Confidence in Management		( - )	
0 (High)	155	(9)	3 (1.9%)
5 (Moderate)	712	(39)	9 (1.3%)
10 (Some)	634	(35)	15 (2.4%)
20 (Little)	49	(3)	3 (6.1%)
30 (None)	6	(<1)	1 (16.7%)
Not recorded	263	(14)	6 (2.3%)
Hazard Analysis Systems		(11)	· (,
In place and documented	1163	(64)	23 (2.0%)
In place and undocumented	209	(11)	4 (1.9%)
In place; document status not recorded	88	(5)	1 (1.1%)
Not in place	113	(6)	6 (5.3%)
Not recorded	246	(14)	3 (1.2%)
Management Food Hygiene Training		,	
Received training and attended	1581/1819	(87)	33 (2.1%)
Basic 6 hour course	826/1581	(52)	23 (2.8%)
Intermediate course	483/1581	(31)	7 (1.5%)
Advanced course	110/1581	(7)	1 (0.9%)
Other recognised	79/1581	(5)	1 (1.3%)
Not specified	83/1581	(5)	1 (1.2%)
No training	41/1819	(2)	1 (2.4%)
Not recorded	197/1819	(11)	3 (1.5%)

Most samples (86%) were obtained from premises with a consumer at risk score 5 (few numbers of customers, 51%) and 10 (intermediate number of customers, 35%) (Table 11). The proportion of cheeses of unsatisfactory quality collected from premises with a very small number of customers was

higher (2.8%) when compared to other premises with larger numbers of customers (1.1%) (p=0.0300).

Most samples (74%) were collected from premises where there was a confidence in management score of 5 (moderate confidence in management/control systems, 39%) and 10 (some confidence in management/ control systems, 35%) (Table 11). Significantly a greater proportion of cheeses of unsatisfactory quality were from premises with high scores (7.3%) (i.e. little or no confidence in the management) compared those with a low score (1.8%) (i.e. some to high confidence in management) ((p= 0.0216) (Table 11).

#### Hazard analysis systems

Eighty percent of samples were collected from premises that had a hazard analysis in place (64% documented, 11% undocumented; 5% documentation status not recorded) (Table 11). Samples collected from premises without hazard analysis systems in place were more likely to be of unsatisfactory microbiological quality (5.3%) compared to those collected from premises with hazard analysis in place (1.9%) (Table 11) (p=0.0308).

#### Food Hygiene Training

The majority of samples (87%) were collected from premises whose managers had received some form of food hygiene training (Table 11). The proportion of samples of unsatisfactory quality were similar from premises whether the manager had received food hygiene training (2.1%) or not (2.4%). However, it should be noted that the proportion of samples taken from premises where the manager had not received food hygiene training is comparatively small compared to those that had trained managers. Therefore no statistical conclusions should be drawn from these results.

#### Discussion

This study has shown that the vast majority (98%) of 1819 retail cheeses made from raw or thermised milk in the UK were of satisfactory or borderline microbiological quality according to criteria in EC Recommendation

2004/24/EC<sup>16</sup>. Likewise, in the following study in 2005 of pasteurised milk cheeses, 98% were found also to be of satisfactory or borderline microbiological quality according to Recommendation 2005/175/EC<sup>17,28</sup>. Only 2% of samples in the present study were unsatisfactory due to *S. aureus* ( $\geq 10^4$  cfu/g, 13 samples), *E. coli* ( $\geq 10^5$  cfu/g, 25 samples), *L. monocytogenes* ( $\geq 10^2$  cfu/g, 1 sample), and the presence of *Campylobacter* spp. in one sample; full investigations were undertaken by the appropriate food authority, manufacturers and the UK Food Standards Agency. Cheeses were also sampled from production premises although in much lower numbers; of 23 batches examined five were of unsatisfactory quality due to high levels of *S. aureus* (3 samples), *E. coli* (1 sample), and *L. monocytogenes* (1 sample).

The Salmonella and L. monocytogenes criteria used in Recommendation 2004/24/EC<sup>16</sup> are the same as the food safety criteria in Regulation (EC) No. 2073/2005<sup>29</sup> on the microbiological criteria for foodstuffs that came into force in January 2006. However, this Regulation contains different criteria for S. aureus in raw ( $\geq 10^5$  cfu/g) and thermised ( $\geq 10^3$  cfu/g) milk cheeses which are applicable only during the manufacturing process when the number of staphylococci is expected to be highest. Additionally where S. aureus levels exceed 10<sup>5</sup> cfu/g the cheese batch has to be tested for staphylococcal enterotoxins as required by food safety criteria and withdrawn or recalled from the market if present<sup>29</sup>. In most cheeses S. aureus levels are highest 2-3 days after production and may reduce significantly during storage. If levels exceed  $10^5$  cfu/g at any point there is a significant risk that S. aureus may produce enterotoxins that will remain in the cheese regardless of the remaining recoverable level of this organism. Although Recommendation 2004/24/EC only deemed levels exceeding 10<sup>4</sup> cfu/g as unsatisfactory, levels exceeding 10<sup>3</sup> cfu/g in hard and semi-hard cheeses on retail sale that have a long shelf life should also be viewed with suspicion due to the likely reduction in staphylococcal levels during storage. There are no criteria for E. coli in cheeses made from raw or thermised milk in Regulation (EC) No. 2073/2005<sup>29</sup> and it's therefore recommended that E. coli O157 be sought for in these cheese types.

*S. aureus* is the commonest cause of mastitis in dairy animals, and appears to be more common in raw goats' and ewes' milk than in cows' milk<sup>30</sup>. In this study significant numbers ( $\geq 10^4$  cfu/g) of *S. aureus* were found more in goats' milk cheese (2%) compared with cows' milk cheese (1%), and almost a third of the isolates contained the genes for staphylococcal enterotoxin production. There is published evidence that a high proportion of isolates of *S. aureus* from both goats' and ewes' milk produce enterotoxins<sup>31,32</sup>, and outbreaks of staphylococcal food poisoning in France and Scotland in 1984-5 were traced to cheese made from raw ewes' milk<sup>12</sup>. An outbreak in Norway in 2003 was associated with a product made with raw cows' milk containing *S. aureus* that subsequently produced sufficient staphylococcal enterotoxin H to cause food poisoning<sup>33</sup>. It should be noted that post-processing contamination by *S. aureus* is also possible through unhygienic handling of the product.

The prevalence of *L. monocytogenes* observed in retail raw milk cheese in the UK in 2004 (0.9%) was similar to that found in Ireland (0.2%; in 2004)<sup>34</sup> and lower than that previously found in the UK (1.4%; in 1995)<sup>35</sup>, in Belgium (46.7%; in 2000-01)<sup>36</sup>, and in Sweden (42%; in 1994)<sup>37</sup>. The serogroups most often causing infection in the UK are serogroups 4b, 1/2a, and 1/2b<sup>38</sup>, with the subtype 4b AFLP I being most common, whereas the predominant serogroup recovered from food isolates in the United Kingdom during 2002 to 2005 was serogroup 1/2a, of which half were AFLP VII (J McLauchlin and K Grant, HPA pers comm). The predominant serogroup of *L. monocytogenes* recovered from the referred cheese isolates was serotype 1/2a, with subtypes 1/2a AFLP VII and IX prevalent. Subtype 4b/V that caused the outbreak of listeriosis in England in 2003 attributed to consumption of butter<sup>39</sup> was recovered from one cheese sample in this study. The low prevalence of serogroup 4b in food isolates compared with clinical isolates has also been observed in other countries<sup>40-42</sup>.

This study has also highlighted contributory factors likely to cause problems with the microbiological quality of cheeses made from raw or thermised milk. According to microbiological criteria within Recommendation 2004/24/EC<sup>16</sup> cheeses were of unsatisfactory quality more frequently if they were: unripened

soft cheese varieties; from premises without hazard analysis system in place; from premises rated as having little or no confidence in management and control systems; from farm shops or markets; stored or displayed above 8°C. Appropriate hygienic measures to avoid contamination from the production environment and appropriate temperature control for soft and ripened cheeses are critical for minimising contamination with and growth of pathogens, such as *L. monocytogenes* and *S. aureus,* in cheeses. Storage of foods must comply with Regulation (EC) No. 852/2004 on the hygiene of foodstuffs<sup>7</sup>, i.e. should not be kept at temperatures that might result in a risk to health.

The UK Advisory Committee on Microbiological Safety of Food in 1995 recommended that cheese made from raw milk from cows and other species be labelled with 'made from raw milk' so that consumers can identify it due to concerns that raw milk may contain organisms such as verocytotoxinproducing *E. coli* (VTEC)<sup>43</sup>. At the time of this study there was still no legal requirement to label raw milk cheese in this way. However, Regulation (EC) No. 853/2004 laying down specific rules for food of animal origin does now require that all cheeses made with raw milk and on retail sale must be clearly labelled with the words 'made with raw milk' so as to inform consumer choice<sup>8</sup>. This requirement does not extend to cheeses made with thermised milk i.e. using milk treated with a lower heat treatment than pasteurisation. In nearly a fifth (18%) of raw milk cheeses sampled in the present study there was no labelling information with the product to enable the purchaser to determine whether the cheese was prepared from raw milk. Raw milk cheeses should be clearly labelled as such at all retail outlets. In addition, vulnerable groups, such as pregnant women, are advised not to consume soft mould ripened cheeses such as Camembert, Brie or chevre (a type of goats' cheese), or others that have a similar rind, and blue cheeses as they may contain L. monocytogenes<sup>15</sup>.

Although risks are attached to the production of cheese from unpasteurised milk, these can be managed provided the cheesemaker is aware of potential hazards and their control. In addition to the EU hygiene regulations that came

into force in January 2006<sup>8-9</sup>, the Specialist Cheesemakers' Association Code of Best Practice is a comprehensive and valuable guide for both cheesemakers and retailers to help minimize microbial food safety hazards<sup>10</sup>.

#### Acknowledgements

The authors would like to thank all the staff in the Environmental Health Departments throughout the UK who collected samples for this study, and all the staff in both HPA, HPA collaborating and non-HPA laboratories who performed the microbiological examinations. Thanks are extended to FSML and LEP, HPA Centre for Infections for typing isolates, to David Lock at LACORS for co-coordinating the participation of Environmental Health Practitioners, to the Regional Food, Water and Environmental Co-coordinators Forum for advice on the sampling protocols, and to Lilian Hucklesby for co-ordinating data entry.

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#### Annex 1: Participating Laboratories and Local Authority Food Liaison Groups

HPA Region	Laboratory Name	Number of Samples
East	Chelmsford	101
	Norwich	130
London	London FWEM <sup>1</sup>	171
South East	Ashford	68
	Brighton	132
	WEMS <sup>2</sup>	103
West Midlands	Birmingham	23
	Coventry	97
	Shrewsbury& Telford	34
	Hereford	22
North West	Chester	52
	Preston	133
	Carlisle	18
North East, Yorkshire & the Humber	Hull	50
	Leeds	32
	Newcastle	39
	Sheffield	65
South West	Bristol	102
	Exeter	35
	Gloucester	35
	Plymouth	18
	Truro	8
East Midlands	Leicester	20
	Lincoln	134
Total		1622

#### Table I: Participating HPA and HPA Collaborating Laboratories and number of samples

1, London Food, Water & Environmental Microbiology Laboratory 2, Wessex Environmental Microbiological Services

#### Table II: Participating Other Laboratories and number of samples

Nation	Laboratory	Number of Samples
Northern Ireland	Belfast City Hospital	63
Scotland	Aberdeen City Council Public Analysts	13
	Edinburgh A & S Services	2
	Glasgow Scientific Services	21
Wales	Cardiff	21
	Carmarthen	85
	Rhyl	15
Total		220

Table III: Participating Food Safety Liaison Groups and number of samples		
Local Authority Food Liaison Group	Number of Samples	
Berkshire	16	
Buckinghamshire	4	
Cambridgeshire	60	

36

25

39

38

21

8

68

49

35

39

24

68

37

68 37

32

29

48

35

58

20 60

12

30

44

63

80 40

22

35

29

35

33

53

34

36

20

40

85

52 68

28

14

35

1842

#### ... .. . . . . . . . . . .

1, London Food Co-ordinating Group

Cheshire

Cumbria

Devon

Dorset

Essex

Durham

East Sussex

Gloucestershire

Greater Manchester

Humberside

ancashire

incolnshire

Merseyside North Yorkshire

Norfolk

Northamptonshire

Nottinghamshire

South West Yorkshire

Oxfordshire

Shropshire

Somerset

Suffolk

Surrey

Staffordshire

Tyne & wear

West Midlands

West of England

West Sussex West Yorkshire

Wiltshire

Total

Wales North Group

Wales South West Group

Northern Ireland Food Group<sup>2</sup>

Scottish Food Enforcement Liaison Committee<sup>3</sup>

eicestershire

Kent

Hampshire & Isle Of Wight Hereford & Worcester

Hertfordshire & Bedfordshire

FCG<sup>1</sup> Greater London NE Sector

FCG Greater London NW Sector

FCG Greater London SE Sector

FCG Greater London SW Sector

Derbyshire

2, Northern Ireland Food Group comprises of the Eastern, Northern, Southern and Western Groups

3, SFELG comprises of Central Scotland, Fife & Tayside, Lothian & Scottish Borders, North Scotland, and West of Scotland