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Assessment of hygienic quality of some types of cheese sampled from retail outlets

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Summary

The authors evaluated the prevalence of *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella* spp. and staphylococcal enterotoxin in 2 132 samples selected from six types of cheese on the basis of recorded consumption in Italy in 2004. In *L. monocytogenes*-positive samples the precise level of contamination was determined. To define the physical-chemical characteristics of the selected natural cheeses, the pH values, water activity and sodium chloride content were determined. The results suggest that blue and soft cheeses (Brie, Camembert, Gorgonzola and Taleggio) are more likely to be contaminated with *L. monocytogenes*. The mean prevalence of *L. monocytogenes* in the six types of cheese was 2.4% (from 0.2% in Asiago and Crescenza to 6.5% in Taleggio), with contamination levels of up to 460 MPN/g. No presence of *Salmonella* spp. and *E. coli* O157 was found in any sample. Staphylococcal enterotoxin was found in 0.6% of the samples examined. Physical and chemical parameter values confirmed that all types of cheese are considered capable of supporting the growth of *L. monocytogenes*. The study confirmed the need to apply effective control at production and sales levels to reduce the probability of contamination by *L. monocytogenes*. This micro-organism can attain high levels of contamination in food products, such as cheeses that have a long shelf-life when associated with difficulties of maintaining appropriate storage temperatures in both sales points and in the home.

Keywords

Asiago, Brie, Camembert, Cheese, Crescenza, Enterotoxin, Food, Gorgonzola, Italy, *Staphylococcus*, *Escherichia coli* O157, *Listeria monocytogenes*, *Salmonella* spp., Safety, Taleggio.

Introduction

Cheese is one of the most popular foods in Italy. In 2004, its 'penetration index' (a parameter that measures the popularity of a product among consumers) was 99.5%, while its value accounted for 11% of the basket of goods and services (21). It is considered a safe food, given that the milk used for production is predominantly pasteurised and is thus free of the most common pathogens responsible for food poisoning (17, 18, 24). However, despite this favourable public health aspect, some cheeses are more susceptible than others to bacterial contamination and spread. The causes include both intrinsic factors (nutrients, water activity, pH, inhibiting factors produced by starter cultures and non-starter micro-organisms, competitive microflora, etc.) and extrinsic factors (microbial quality of raw milk, production phases, ripening and packaging conditions, etc.) (34). Studies on various types of cheese have identified that microbiological contamination can generally be attributed to the use of raw milk and unsuitable production and ripening environments (7, 8, 12, 14, 15, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 38).

To determine the real risk for consumer health in relation to cheese consumption, the

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European Union issued recommendations in 2003 for coordinated programmes to ensure the official control of foodstuffs.

These programmes first examined cheeses made with raw and heat-treated milk and then cheeses made with pasteurised milk (9, 11). European Commission Regulation (EC) No. 2073/2005, which covers the micro-biological criteria for ready-to-eat products, established a maximum level for *Listeria monocytogenes* of 100 cfu/g throughout the shelf-life (4). In the absence of scientific evidence that contamination is contained within the prescribed levels, the regulations oblige food business operators to conduct studies of contamination levels throughout the shelf-life of a product. Without such studies, the criterion of 100 cfu/g cannot be applied, resulting in 'zero tolerance' of *L. monocytogenes*. The same regulations also require zero tolerance of *Salmonella* spp. and *Escherichia coli* O157 (4).

Data from European control programmes and the Rapid Alert System for Food and Feed (RASFF) indicate that microbiological contamination of cheese is widespread in Europe (4, 5).

The 2005 European Food Safety Authority (EFSA) report on pasteurised milk cheeses found *L. monocytogenes* in 0.5% of samples (42 of 8 062 examined), *Salmonella* spp. in 0.056% (2 of 3 519) and *E. coli* O157 in 0.17% (5 of 2 876). All positive samples came from soft or semi-soft cheeses. Staphylococcal enterotoxin was found in 0.34% of samples (3 of 884) – two semi-hard cheeses and one ripened soft cheese (2).

The 2006 EFSA report included 10 262 samples of raw and pasteurised milk cheeses of various types (ripened and unripened, with and without mould, soft, semi-soft and hard cheeses) taken across Europe (except Germany), found a level of positivity of 0.7% for *L. monocytogenes* (22).

In 2006, of 1 696 samples examined in Germany, 19.3% were found to contain *L. monocytogenes*; in 19.3% of the positive samples, the contamination level was below the limit of 100 cfu/g and in 0.3%, the *L. monocytogenes* concentration was higher that the regulatory limit.. In Italy, *L. monocytogenes* was isolated in 1.4% of the 3 861 cheese samples examined (5).

The study presented here evaluated the microbial quality and hygiene standards associated with the production and handling of selected cheeses among the more popular cheeses at national level (22).

The levels of contamination by *L. monocytogenes*, *E. coli* O157:H7, *Salmonella* spp. and staphylococcal enterotoxin were determined. The degree of association between some risk factors measured at the retailers and the frequency of bacterial contamination were calculated. The factors identified described the cleanliness of equipment and good hygienic practices of the sales people.

Materials and methods

Selection of cheeses and sales points

Between March 2005 and October 2006, samples of six types of cheeses, namely:

- Asiago
- Brie
- Camembert
- Crescenza
- Gorgonzola
- Taleggio

were taken from sales points in the Abruzzo region. These cheeses are among the most popular in Italy and also some of those most susceptible to *L. monocytogenes* contamination.

Sales outlets were chosen at random from the Italian 'Yellow Pages' (SEAT SpA, Turin) after classification by province on the basis of the number of residents in the four Abruzzi provinces (1, 23), and by outlet type (large- and small-scale retail) on the basis of purchases in Italy in 2004 (22). A total of 390 sales points were selected in the four provinces as follows:

- 91 in L'Aquila
- 117 in Chieti
- 91 in Pescara
- 91 in Teramo.

However, only 381 were involved in the sampling (Table I); for various reasons (limited

sale of products, closure of business, etc.) the cheeses to be sampled could not be found in nine sales points.

As shown in Table II, a total of 2 132 samples were taken as follows:

- Asiago: 449
- Brie: 300
- Camembert: 178
- Crescenza: 437
- Gorgonzola: 444
- Taleggio: 234.

The sample taken was about 500 g, although smaller samples (lack of sufficient product at sales point) were not excluded, as long as there was an adequate amount for *L. monocytogenes* testing. All samples were only tested for *Listeria*. A sample sheet was completed upon sampling to collect information on the sales point (name, address, type), product (packaging type, storage method: display counter dividers, temperature) and shop assistants' clothing, level of hygiene and use of instruments. Samples were taken from the sales point to the laboratory by refrigerated transport (between 0°C and +4°C).

Risk factors for the assessment of *Listeria monocytogenes* contamination

To determine the contribution of environmental conditions to the microbial standards of cheese sold at retail outlets, the cleanliness of equipment and tools used by sales staff and good practices adopted by them were monitored.

In particular, the following were checked and recorded:

- location of the product (refrigerated bench, etc.)
- presence of other types of food in the same compartment
- presence of partitions between cheese and other foods
- pre-packaged ready product or product wrapped at point of sale
- protection of the cut surface and type of packaging materials used
- types of tools used for portioning and their management.

Laboratory tests

Microbiological testing of Taleggio, Brie, Camembert and Gorgonzola was conducted on the cheese, including the rind. This was designed to simulate the effects of incomplete removal, as often happens in home environments, and also in consideration of the guidelines of the *Consorzio di Tutela del Taleggio* (Consortium for the Protection of Taleggio), which considers the rind to be edible after scraping (7).

All samples (2 132) were tested for *L. monocytogenes*, 2 127 for *E. coli* O157, 2 126 for *Salmonella* spp. and 2 107 for staphylococcal enterotoxin. A polymerase chain reaction (PCR) Bax system was used to detect *L. monocytogenes* (37), *Salmonella* spp. (3) and *E. coli* O157 (19). The most probable number (MPN) method (36) was used for quantification of samples positive for *L. monocytogenes*, and the enzyme-linked

Table I
Sales points used for cheese sampling

Sales point	Chieti	L'Aquila	Pescara	Teramo	Total
Discount store	7	8	4	2	21
Hypermarket	13	0	10	3	26
Supermarket	63	50	45	55	213
Total large-scale retail	83	58	59	60	260 (68.2%)
Minimarket	11	6	8	23	48
General stores	21	8	26	18	73
Total small-scale retail	32	14	34	41	121 (31.8%)
Total large- and small-scale retail	115	72	93	101	381

Table II
Cheese samples purchased per sales point

Sales point	Asiago	Brie	Camembert	Crescenza	Gorgonzola	Taleggio	Total
Discount store	27	29	11	23	29	6	125
Hypermarket	16	46	78	22	16	32	210
Supermarket	281	204	85	281	284	230	1 365
Total large-scale retail	324	279	174	326	329	268	1 700 (79.7%)
Minimarket	52	12	3	46	47	23	183
General stores	73	9	1	65	68	33	249
Total small-scale retail	125	21	4	111	115	56	432 (20.3%)
Total large- and small-scale retail	449	300	178	437	444	324	2 132

fluorescent assay (EFFA) method (Vidas, BioMérieux) was used to detect staphylococcal enterotoxin.

The pH was determined using a potentiometer (20), the concentration of sodium chloride was determined by titration (1) and water activity was measured using the dew point method (21).

Statistical analysis

Prevalence was calculated using a 95% Bayesian confidence interval.

Any association between the prevalence of bacterial contamination and the variables considered was evaluated separately with the χ^2 test and cumulatively using logistic regression.

The following were considered as variables with respect to the hygiene of the product:

- type of sales point (small retail or large retail)
- display counter dividers, protective wrapping of product
- portioning on purchase and sampling method (performed by shop assistant or taken directly by researcher from the display counter).

Results

Laboratory tests

Tests for *L. monocytogenes* were negative in Camembert (0/178) and positive in Asiago (1/449), Brie (3/300), Crescenza (1/437), Gorgonzola (21/444) and Taleggio (21/324). The percentage of contaminated samples ranged from 0.2% (Asiago and Crescenza) to 6.5% (Taleggio) (Table III). Contamination levels were up to 24 MPN/g for 44 of the 47 positive samples, while three samples of Brie and Taleggio showed values that ranged between 110 and 460 MPN/g (Table IV) (Fig. 1).

Tests for *Salmonella* spp. and *E. coli* O157 were negative (Table V). Staphylococcal enterotoxin was not detectable in Crescenza (0/432) or Taleggio (0/320), but was found (>1 ng/ml) in Asiago (1/442), Brie (2/299), Camembert (1/178) and Gorgonzola (9/436) (Table VI).

Table VII provides the results for water activity, Table VIII give pH values and Table IX indicates sodium chloride values.

Statistical analysis

The χ^2 test did not reveal any significant correlation between positivity for *L. monocytogenes* and three of the potential risk factors considered, namely:

- type of sales point
- presence of display counter dividers
- product wrapping.

There was a low statistical significance for correlation with product portioning ($\chi^2 = 4.06$; $p = 0.04$) and for sampling performed by the shop assistant ($\chi^2 = 4.46$; $p = 0.03$).

No significant correlation was found on logistic regression between the five variables studied and the positive levels of *L. monocytogenes* (Table X).

Table III
Results of tests for *Listeria monocytogenes* in the cheese samples examined, with confidence limits

Cheese	Samples	Positive	Positive (%)	Lower confidence limit 95%	Upper confidence limit 95%
Asiago	449	1	0.2%	0.1%	1.2%
Brie	300	3	1.0%	0.4%	2.9%
Camembert	178	0	0.0%	0.0%	2.0%
Crescenza	437	1	0.2%	0.1%	1.3%
Gorgonzola	444	21	4.7%	3.1%	7.1%
Taleggio	324	21	6.5%	4.3%	9.7%
Total	2 132	47			

Table IV
Listeria monocytogenes quantification in cheese samples that gave positive results

Cheese	Positive samples	Lowest value found (MPN/g)	Highest value found (MPN/g)
Asiago	1	<0.36	<0.36
Brie	3	<0.36	110
Camembert	0	0	0
Crescenza	1	<0.36	<0.36
Gorgonzola	21	0.36	9.3
Taleggio	21	<0.36	460
Total	47		

MPN most probable number

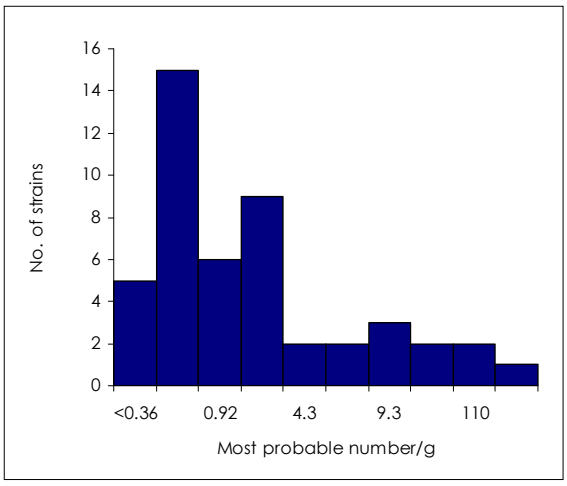

Figure 1
Listeria monocytogenes contamination levels in positive samples

Discussion

The representativeness of the samples taken for the situation in the Abruzzi was guaranteed in this study by sample stratification and sampling over a sufficient period of time to account for any seasonal variations. The results revealed that the prevalence of *Salmonella*, *E. coli* O157 and staphylococcal enterotoxin contamination was similar to that found during official European controls in 2005 and 2006 (6, 7). In contrast, the prevalence of *L. monocytogenes* in Gorgonzola (6.5%) was higher than that found in Italy during the 2002-2003 monitoring programmes for blue cheeses (2.2%) (14). The prevalence of this pathogen in both Gorgonzola and Taleggio was considerably higher than the general situation of Italian cheeses, as revealed by the results of the 2004 coordinated programmes for the official control of foodstuffs (1.8% positive samples) (13) and the 2006 EFSA report (1.4% positive samples) (6).

The high prevalence found in this study in comparison to results of official controls during the same period may be due to a different sampling target (cheeses at high risk of *Listeria* contamination in our sample, against sampling of all cheeses in the official controls)

Table V
Results of tests for *Salmonella* spp. and *Escherichia coli* O157, with a 95% upper confidence limit

Cheese	Samples (no. of samples examined for *Escherichia coli* O157)	Positive	Upper confidence limit 95%
Asiago	449	0	0.7%
Brie	299 (300)	0	1.0%
Camembert	178	0	1.7%
Crescenza	436	0	0.7%
Gorgonzola	441	0	0.7%
Taleggio	323	0	0.9%
Total	2 126 (2 127)		

Table VI
Results of tests for staphylococcal enterotoxin in the cheese samples examined, with confidence limits

Cheese	Samples	Positive	Positive (%)	Lower confidence limit 95%	Upper confidence limit 95%
Asiago	442	1	0.2	0.1%	1.2%
Brie	299	2	0.7	0.2%	2.4%
Camembert	178	1	0.6	0.1%	3.1%
Crescenza	432	0	0.0	0.0%	0.7%
Gorgonzola	436	9	2.1	1.1%	3.8%
Taleggio	320	0	0.0	0.0%	0.9%
Total	2 107	13			

Table VII
Water activity content in cheese samples examined

Cheese	Samples	Mean	Minimum	Maximum
Asiago	68	0.967	0.926	0.988
Brie	53	0.978	0.936	0.996
Camembert	34	0.980	0.965	0.996
Crescenza	56	0.985	0.928	1.000
Gorgonzola	62	0.964	0.919	0.994
Taleggio	59	0.963	0.921	0.995
Total	332			

Table VIII
pH values in cheese samples examined

Cheese	Samples	Mean	Minimum	Maximum
Asiago	369	5.715	5.209	7.660
Brie	191	6.862	5.370	8.176
Camembert	66	6.827	5.500	7.930
Crescenza	359	5.526	4.900	7.270
Gorgonzola	362	6.629	4.989	7.996
Taleggio	219	6.088	5.374	8.033
Total	1 566			

Table IX
Sodium chloride content (g/100 g) in cheese samples examined

Cheese	Samples	Mean	Minimum	Maximum
Asiago	69	1.97	0.84	2.83
Brie	53	1.89	0.99	2.82
Camembert	32	1.71	1.16	3.24
Crescenza	57	0.97	0.59	1.16
Gorgonzola	63	1.94	1.15	4.26
Taleggio	60	2.66	1.85	3.42
Total	334			

Table X
Logistic regression of storage and sales procedures: estimated values for the parameters considered (maximum probability) for *Listeria monocytogenes*

Parameter	Estimated value	Standard deviation	χ^2	$p>\chi^2$
Portioning on purchase	0.072	0.405	0.031	0.859
Presence of display counter dividers	–0.655	0.501	1.712	0.191
Product served	–0.677	0.493	1.890	0.169
Presence of wrapping	0.040	0.410	0.009	0.922
Sold in small-scale retail	–0.478	0.504	0.900	0.343

and the higher number of samples taken (2 132 in this study, against 543 in the official controls). Furthermore, three samples in this study were found to have a contamination level that ranged between 100 MPN/g and 460 MPN/g, whereas no case of contamination above 100 cfu/g was found in the official controls performed.

With respect to the retail variables studied, the lack of any significant difference in samples positive for *L. monocytogenes* between cheeses stored and handled with good hygiene practice and those handled using non-hygienic procedures leads to the supposition that the greatest risk of contamination of cheeses can be found during the pre-sale production phases (34).

The absence of *Salmonella* spp. and *E. coli* O157 contamination in this study, in contrast with the high prevalence of *L. monocytogenes* contamination, was probably due to the different ecologies of these three bacteria, namely: *L. monocytogenes* is predominantly an environmental contaminant, whereas *Salmonella* spp. and *E. coli* O157 are introduced with the raw materials.

This aspect of *Listeria* ecology has been demonstrated in the literature (25) revealing, through analyses of Gorgonzola rind microflora, that contamination occurs mainly during the ripening phase and that, as a safety measure, manufacturers and health education boards would be advised to encourage removal of the rind before consumption.

The chemical and physical analysis of our cheese samples revealed the properties typical of these products (30), which fall within the conditions that favour pathogen contamination and replication, particularly of *L. monocytogenes* (pH 4.4-9.4, water activity 0.90-0.93 and sodium chloride ≤10%) (31).

The variations did not enable a valid risk analysis for each type of cheese, but required each individual commercial product to be considered separately.

The increase in the authorised contamination level to 100 cfu/g established by Commission Regulation (EC) No. 2073/2005 led to a raised level of risk for the consumer that was 2 500 times higher than was the case with the previous limit (no *Listeria* in 25 g). This becomes particularly serious considering that

all the cheese samples examined had chemical and physical parameters favourable for the survival and growth of *L. monocytogenes* at the refrigeration temperature at which they were stored in the retail phase. Furthermore the dose-response is influenced not only by the contamination level but also the virulence of the strain and the host's immune status (10).

The contamination level that may be reached by the time of consumption may thus be even greater than the already high value accepted by the regulations. It follows that given the chemical and physical properties of the cheeses examined, further microbiological evaluation is necessary for this type of food and enhanced controls may be warranted during the production phases.

Further confirmation of the need to review this aspect is provided by epidemiological data which demonstrate that in Europe, between 1999 and 2001, about half of the outbreaks and sporadic cases of listeriosis in humans were associated with the consumption of cheeses produced from raw and pasteurised milk (28), and that in France and other European countries, the incidence of human listeriosis increased in 2006 (16).

Conclusions

Findings of *L. monocytogenes* in cheeses made in Italy and marketed in both European and non-European countries are frequently reported through the European Union's food safety alert system. In view of these reports, the level of attention to *L. monocytogenes* contamination of these products needs to be increased throughout the production chain, at least up to the retail sale stage.

It is not yet possible to establish the true risk for consumers because data relating to the presence of *L. monocytogenes* are insufficient and incomparable. Moreover, the small number of positive results relates to cheeses that are not reported in the alerts and to those indicated in the literature as 'at-risk' products due to their chemical and physical characteristics and the manufacturing technologies used. The level of attention to *L. monocytogenes* contamination of these products needs to increased throughout the production chain, or at least up to the retail sale stage.

The prevalence of *L. monocytogenes* and the contamination levels found in four of the six cheeses studied (Brie, Camembert, Gorgonzola and Taleggio) suggest that the microbiological criteria laid down by Regulation (EC) No. 2073/2005 for these products should be reconsidered, as the values of the chemical and physical parameters found in these cheeses favour the survival and growth of *Listeria*. Moreover, the ability of *Listeria* to survive and multiply even at refrigerated temperatures during its shelf-life could expose consumers to unacceptable levels of contamination, especially in the case of the vulnerable consumers in the highest risk categories. Furthermore, some of the cheeses examined have long shelf-lives which, in view of the difficulty of guaranteeing that correct storage temperatures are maintained, especially in the home, could lead to the presence of high levels of *L. monocytogenes* in the product in a short period of time.

Despite the low levels of *L. monocytogenes* contamination reported in the literature and confirmed in our study, there has been an increase in cases of human listeriosis in Europe in the last two years. This suggests the need to investigate the mechanisms involved in transmission of the infection, to establish the exact bacterial load in food at the time of consumption and to identify the specific production stage that most needs reinforced controls. Not all *L. monocytogenes* are equally pathogenic for humans and further studies on the pathogenicity of the *L. monocytogenes* strains isolated are necessary if we are to truly understand the epidemiology of this pathogen.

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