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Identification of the risk factors associated with cheese production to implement the hazard analysis and critical control points (HACCP) system on cheese farms

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ABSTRACT

The purpose of this paper was to evaluate, by statistical analyses, risk factors on cheese farms that can influence the microbial contamination of their products. Various assessment tools, such as cheese production questionnaires, food handlers' knowledge testing, and hygiene assessment system surveys, were used on 39 cheese farms on the island of Gran Canaria, Spain. The microbiological status of 773 raw milk and cheese samples from the cheese farms was assessed by enumerating total viable counts and 4 pathogens: *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Salmonella* spp. The results revealed that the highest contamination by *Staph. aureus* (4.39%, $>10^5$ cfu/mL) was found in milk, and the highest contamination by *E. coli* (5.18%, $>10^3$ cfu/mL) was found in cheese. Very few samples (0.52%) were contaminated by *L. monocytogenes* or *Salmonella* spp. The factors associated with any tested microorganism were "handling," "knowledge," and "type of milk." Subsequently, multidimensional logistic analysis for contamination by *E. coli* showed an independent association for factors "cleaning and disinfection test" and "type of milk." The probability of total aerobic contamination of milk increased with lower hygiene assessment system survey scores. These results emphasize the need to apply and maintain good hygiene practices, and to study risk factors to prevent contamination and bacterial growth. Further research is required in other areas with different cheese farm types to reinforce the validity of these results.

Key words: cheese farm, risk factor, hazard analysis and critical control points (HACCP), pathogenic microorganisms in cheese

INTRODUCTION

Cheese making is a major industry worldwide, and much of it is still practiced on a relatively small scale, which accounts for the rich diversity of available cheeses (Fox and McSweeney, 2004). Canary cheeses form part of the cultural heritage of the Canary Islands, the region with the highest production and consumption of goat milk cheeses in Spain. Cheese production in the region is widespread, with about 391 dairies (16,247 and 782 t/yr of goat and ewe milk cheese, respectively, of which 52.4% is raw milk cheese); 176 cheese farms are located on Gran Canaria Island (producing 4,300 t/yr of goat milk cheese; Fresno et al., 2012), where approximately 78% of cheese is produced on cheese farms and the rest in cheese factories (Fresno et al., 2012). These data suggest consumer preference for artisanal cheese. Both artisanal and industrial cheeses are an essential component of the Canary diet and economy but are potentially hazardous products if processed under noncompliant conditions (Karaman et al., 2012).

Because food hazards exist for such cheeses, it is important to introduce a system that ensures safety along the food chain. Hazard analysis and critical control points (**HACCP**) is a food safety management approach that, in the last few decades, has been used by national governments, and is an international strategy adopted to reduce the prevalence of foodborne disease (Bas et al., 2007). Food hygiene is a primary and widespread goal for the food industry, and it includes

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the hygienic design and engineering of installations and facilities, engineering of equipment, and integration of components and maintenance (Betta et al., 2011).

Today, the implementation of food safety management systems into small production businesses is very difficult; such systems are considered excessively complicated given the huge amount of documentation involved and the need for additional economical resources (Le et al., 2014). For these reasons, implementing effective tools into HACCP would allow the management of hygienic processing and its traceability. Practical experience and a review of the food safety literature indicate that success in developing, installing, and monitoring a HACCP system relies on the appropriate combination of managerial, organizational, and technical hurdles in enterprises (Bas et al., 2007). However, small and medium-sized enterprises may feel that the difficulties of implementing HACCP are potentially insurmountable (Route, 2001). The potential barriers to implement HACCP include lack of expertise, legal requirements, financial constraints, and attitudes (Taylor, 2001; Walker et al., 2003). Nevertheless, these difficulties do not correspond to those found on cheese farms of Gran Canaria, where the reasons for not fully implementing HACCP could be excessive document-processing tasks and the complexity of the self-control system itself. However, all parties (cheese farmers and Canary Health Service of The Autonomous Government of the Canaries, Gran Canaria Island, Spain) are aware of the importance of HACCP as a preventive model in food safety.

Determining the factors linked to production and food handlers could help producers implement HACCP and reduce the likelihood of microbiological hazards, such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus* in end products. These pathogens, which are found in raw milk (D'Amico and Donnelly, 2010; Mee et al., 2012), can persist in the cheese-making environment and contaminate cheese during production (Ahmed et al., 2000; Hill and Warriner, 2011). To our knowledge, no other similar studies have evaluated risk factors related to microbiological quality on cheese farms. Therefore, the purpose of this study was to identify the main risk factors that relate to pathological microorganisms isolated in milk and cheese from Gran Canaria to facilitate HACCP implementation.

MATERIALS AND METHODS

Cheese Farms

In this study, 176 cheese farms were identified on the Island of Gran Canaria (Canary Islands, Spain), which were all subsequently contacted by telephone or e-mail; 39 agreed to participate. Interviews were conducted with food handlers, and cheese samples were collected for 1 yr. Each cheese farm was visited by staff trained in HACCP and prerequisite programs to conduct the 3 face-to-face questionnaires used. The 39 cheese farms that participated in this study fulfilled the following requirements: (1) they were authorized by the Canary Health Service of The Autonomous Government of the Canaries to produce and sell cheese; (2) all operated food safety management (HACCP); (3) they produced between 10 and 1,000 L of milk per day; all made fresh cheese; (4) they collected milk from their own cattle and were not allowed to buy milk from other farms; and (5) they employed between 2 and 4 workers

Questionnaire Design

To determine risk factors, 3 questionnaire and tools were used: production questionnaire, food handlers' hygiene knowledge, and a hygiene assessment system (**HAS**) score, which were related to the microorganisms isolated in milk and cheese. The authors applied the principles of this interrelated framework to design questions to assess the knowledge of the food safety risks associated with artisanal cheese making (Le et al., 2014). The risk analysis framework is a science-based structured policy development tool used by risk managers to reduce risks to acceptable levels. It incorporates 3 interrelated components: risk assessment, risk management, and risk communication (Cahill, 2003; WHO/FAO, 2006; Gunn et al., 2008).

The structured questionnaires about cheese production, food handlers' hygiene knowledge, and a HAS score, were conducted during the first 3 visits to the cheese farms. Managers were interviewed first, and then workers. Research team members read each question aloud during the interview and then asked questions to ensure clarity (Karaman et al., 2012). Figure 1 shows all the steps undertaken in this study.

Questionnaire Contents

Pretesting. Before conducting the questionnaire, questions were pretested with a production worker, a quality assurance manager, and a cheese farm manager during an on-site visit to a small cheese farm. Pretesting was supplemented by a tour of the small cheese farms, which helped researchers become familiar with the major processing steps of cheese making, labeling, and cheese sales (Le et al., 2014). The ultimate purpose was to gain the interviewees' trust.

Production Questionnaire. A semi-closed questionnaire on cheese production was conducted by

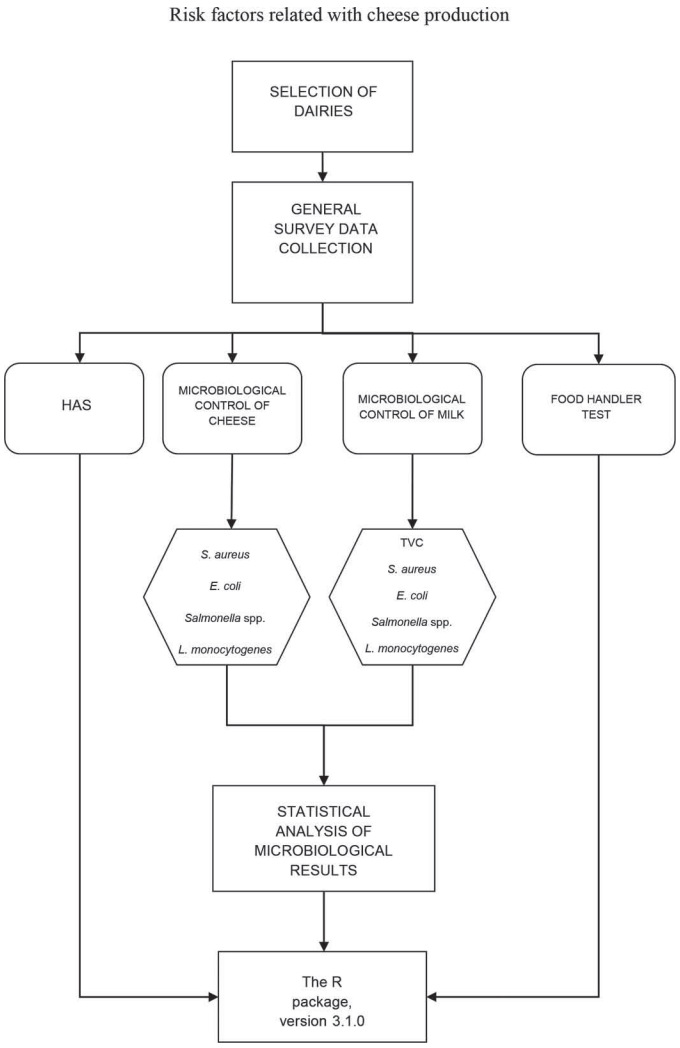

Figure 1. Flowchart of all the steps undertaken in the present study. HAS = hygiene assessment system; TVC = total viable count.

researchers with the manager of each cheese farm. Information was collected on all cheese-making aspects: producer's personal data, cheese ingredients, production and ripening, facilities and utensils, cleaning and disinfection operations, and the self-control system applied on cheese farms. From these data, an attempt was made to acquire information on the technological aspects used to produce artisanal cheese and a comparison was subsequently made among all the participating cheese farms.

Food Handler Questionnaire. Questions in the food handler questionnaire were designed to obtain information on food handlers' knowledge about different aspects of cheesemaking, such as the microbiology of cheese, hygiene practices on the cheese farm, HACCP, and cleaning programs. This questionnaire contained 40 multiple-choice questions, each with 3 possible answers. To reduce the possibility of food handlers selecting the correct answer by chance, the multiple-choice answers included "do not know" (Walker et al., 2003).

Each cheese farm was visited by researchers to conduct face-to-face interviews and was completed individually with no discussion with other personnel. Researchers reviewed the answers individually and explained any incorrect answers. Before starting the interviews, participants were informed about the purpose of the research and were assured that their identities would not be revealed (Walker et al., 2003).

HAS Survey Scores. Assessment of the overall hygiene status on cheese farms by HAS survey can provide useful management data about whether cheese farms are improving or whether, despite still meeting legal requirements, they fail to maintain previously high standards (García and Jukes, 2008). The HAS survey score was designed by Carrascosa (2010) and has been adapted from the HAS survey score used in abattoirs in the United Kingdom (García and Jukes, 2008), and in food vending machines (Raposo et al., 2015) and breweries (Raposo et al., 2013) in Spain. It consists of 7 parts: (I) outside zone, evaluated by 5 questions; (II) inside zone, 10 questions; (III) utensils and equipment-maintenance, 7 questions; (IV) quality of raw and ready-to-eat food, 4 questions; (V) personal hygiene (employee's hygiene practices), 14 questions; (VI) cleaning and disinfection programs, 13 questions; (VII) cheese making, 8 questions; and (VIII) legal and administrative requirements, 3 questions.

Researchers were responsible for completing the assessment by awarding each aspect a mark according to previous knowledge and a predetermined scale. We considered a value of 40 out of 100 as the excluding minimum. Below this value, it would not be possible to implement a HACCP system for serious deficiency in any area represented by the survey headings, and it would be necessary to take immediate measures to reduce the risk to public health.

Microbiological Analysis

Microbiological criteria provide guidance for the acceptability of foodstuffs and their manufacturing, handling, and distribution processes. The use of microbiological criteria (food safety and process hygiene) should form an integral part of implementing HACCP-based procedures and other hygiene control measures [Regulation (EC) No. 2073/2005; European Commission, 2005]. The 4 microbial groups observed herein were considered potential pathogens to human health.

Once the interviews were completed, cheese and milk samples were collected for 1 yr from the 39 cheese farms on Gran Canaria (33 made cheese using raw milk and

6 from pasteurized milk). In all, 387 raw milk samples (mixed milk, different species from the milk tank) were analyzed for *E. coli*, *S. aureus*, *Salmonella* spp., *L. monocytogenes* and total viable counts (**TVC**); 386 cheese (2–45 ripening days) samples were analyzed for 24 h after being removed from cheese farms. Furthermore, 82 milk samples were analyzed only for TVC. Nonetheless, TVC was not determined in cheese because this count is not indicative of contamination in cheese. Table 1 shows all samples information for each cheese farm.

For the microbial analysis, 25 g of cheese and 10 mL of milk were analyzed, except for the *L. monocytogenes* and *Salmonella* spp. samples, for which 25 mL was used. Decimal dilutions in peptone water solution (0.85% NaCl with 0.1% peptone; Cultimed, Barcelona, Spain) were used for microbial enumeration purposes.

Each sample was analyzed in duplicate in appropriate media; TVC were determined in plate count agar (Cultimed) and incubated at 31°C for 72 h (Pascual and Calderón, 2002); *Staph. aureus* by Baird Parker + Rabbit Plasma Fibronogen agar (bioMérieux, Marcy-l'Etoile, France; ISO 6888–2; ISO, 1999), incubated at 37°C for 24 to 48 h. *Escherichia coli* was identified by Coli ID agar (bioMérieux; AFNOR, 2014), incubated at 37°C for 24 to 48 h.

To identify *Listeria monocytogenes*, the VIDAS LMO2 method (bioMérieux) was followed, and confirmation was obtained by spreading in Ottaviani-Agosti agar and API Rapidec Mono (bioMérieux) (AFNOR, 2010). The method used to identify *Salmonella* spp. was VIDAS Easy SLM (bioMérieux) (AFNOR, 2008), and confirmation was obtained by ChromID Salmonella (bioMérieux), incubated at 37°C for 24 h, and by the API20 E test (bioMérieux).

Microbiological Criteria

Tests were conducted in accordance with EU Regulation No. 853/2004 (European Commission, 2004) for milk and Commission Regulation (EC) No. 1441/2007 (European Commission, 2007) for cheese (Table 2). Nevertheless, we decided to apply the same microbiological criteria to both samples as a quality parameter. Table 2 shows the microbiological criteria according to above-mentioned European Union Regulations, and the criteria applied in this study when not cited in the regulations.

Classification and Description of Factors for the Risk Analysis

The analyzed data from each cheese farm, and several factors relating to cutting and handling formed cheese curds, salting, cheese ripening, labeling with milk and cheese, and microbiological quality, were considered. These risk factors were as follows:

- Location ("zone"): geographical location of the cheese farm; that is, coast, midlands, and summit areas and the township of Moya, where a Protected Designation of Origin Queso Flor de Guía is produced.
- Daily cheese production ("production") including fresh and cured cheese: <10 kg/d; 10–50 kg/d; >50 kg/d.
- Type of cheese produced ("type of cheese"), fresh (<7 d), soft (7–25 d), and semi-cured cheese (25–45 d), following BOE (2006) on standards for cheese and melted cheese.
- Degree of handling during cheese making ("handling"). This category is based on the level of automation of dairies: low = dairies with pasteurizers, tanks are studded with automatic presses, and there are salting and ripening chambers; medium = dairies with medium-sized curd vats (100–200 L) with no mechanical agitation, pneumatic cheese press, and with or without ripening presses; high = dairies with small tanks or plastic containers used to coagulate milk with no heating possibility for coagulation. Cheeses are drained and pressed by hand.
- Food handlers' knowledge test ("knowledge test"): the number of failures obtained in the test.
- Cleaning and disinfection knowledge test ("CD test"): the number of failures obtained in the cleaning and disinfection section of the food handlers' knowledge test.
- HAS survey score ("HAS"): survey result of the hygienic assessment of dairies.
- Major milk-producing species: goat, ewe, cow, and mixtures of 2 or 3 of these.

Statistical Analysis

On each cheese farm, the number of contaminated milk products followed a binomial distribution, whose parameters were N (sample size of milk products) and p (probability of contamination of a milk product on that cheese farm). We assumed that parameter p would depend on a set of risk factors. When such dependency was modeled by means of the logistic function, the resulting model would be called a binomial logistic regression model.

Each of the factors shown in Table 3 determined a classification of cheese farms. In each of these groups of dairies, the contamination rates by *E. coli* and *Staph. aureus* and the TVC plate counts were obtained by

Table 1. Risk factors analyzed in each cheese farm and number of samples collected per cheese farm[1]

Farm	Zone	Production (kg/d)	Type of cheese	Number of samples				Handling	Animal species	Heat treatment	Knowledge test	CD test	HAS
				Raw milk	Semi cured	Soft	Fresh						
12	Coast	10–50	Semi-cured, 20–45 d	5	5	0	0	Medium	Goat and ewe	Raw	8	5	57
13	Coast	>50	Semi-cured, 20–45 d	1	1	0	0	Minimum	Goat	Raw	5	1	53
20	Coast	>50	Fresh cheese, <7 d	16	6	0	10	Medium	Goat and ewe	Raw	7	2	60
21	Coast	>50	Semi-cured, 20–45 d	2	2	0	0	High	Goat and ewe	Pasteurized	6	4	60
22	Coast	>50	Semi-cured, 20–45 d	8	8	0	0	High	Goat	Pasteurized	11	5	67
23	Coast	10–50	Semi-cured, 20–45 d	17	17	0	0	Minimum	Goat and ewe	Raw	2	0	62
24	Coast	10–50	Semi-cured, 20–45 d	16	15	0	0	Minimum	Goat and ewe	Raw	4	2	54
25	Coast	>50	Semi-cured, 20–45 d	8	6	0	2	High	Goat	Raw	10	3	75
26	Coast	10–50	Fresh cheese, <7 d	8	3	0	5	Medium	Goat	Raw	3	3	58
28	Coast	>50	Soft cheese, 7–20 d	8	0	0	3[2]	Minimum	Goat and cow	Raw/ Pasteurized	9	3	57
29	Coast	10–50	Soft cheese, 7–20 d	8	0	5	8	Minimum	Goat and ewe	Raw	5	1	63
30	Coast	>50	Fresh cheese, <7 d	15	3	0	14	Medium	Goat	Pasteurized	5	2	68
31	Coast	>50	Fresh cheese, <7 d	17	4	0	13	High	Goat and ewe	Raw	9	4	65
32	Coast	>50	Soft cheese, 7–20 d	8	0	0	8	Medium	Goat and ewe	Raw	11	4	57
33	Coast	>50	Soft cheese, 7–20 d	17	1	0	0	Medium	Goat and ewe	Raw	12	4	57
1	Midlands	1–10	Semi-cured, 20–45 d	8	8	0	0	Minimum	Goat	Raw	12	5	39
2	Midlands	10–50	Semi-cured, 20–45 d	8	8	0	0	Medium	Goat and cow	Raw	7	3	65
6	Midlands	>50	Semi-cured, 20–45 d	8	8	0	0	Minimum	Goat and ewe	Raw	9	4	39
7	Midlands	>50	Semi-cured, 20–45 d	17	17	0	0	Medium	Goat and ewe	Raw	13	3	61
8	Midlands	10–50	Semi-cured, 20–45 d	17	17	0	0	Medium	Goat and ewe	Raw	4	1	62
9	Midlands	10–50	Semi-cured, 20–45 d	8	8	0	0	Medium	Goat	Raw	4	2	62
10	Midlands	>50	Fresh cheese, <7 d	8	2	0	6	High	Goat	Pasteurized	2	0	72
11	Midlands	>50	Fresh cheese, <7 d	12	0	0	13	Minimum	Goat, ewe, and cow	Raw	4	0	48
16	Midlands	>50	Fresh cheese, <7 d	8	0	0	8	High	Cow	Pasteurized	10	5	66
17	Midlands	1–10	Fresh cheese, <7 d	8	0	0	8	Minimum	Goat and cow	Raw	12	5	54
18	Midlands	10–50	Semi-cured, 20–45 d	15	16	0	0	Medium	Goat	Raw	7	3	61
19	Midlands	10–50	Semi-cured, 20–45 d	8	8	0	0	Medium	Goat	Raw	10	4	60
27	Midlands	10–50	Semi-cured, 20–45 d	15	16	0	0	High	Goat and ewe	Raw	5	2	63
36	Midlands	10–50	Fresh cheese, <7 d	5	0	0	6	Medium	Goat and ewe	Raw	5	2	48
14	Summit	10–50	Semi-cured, 20–45 d	8	8	0	0	Medium	Goat	Raw	5	2	68
15	Summit	1–10	Semi-cured, 20–45 d	8	8	0	0	Minimum	Goat	Raw	12	5	29
34	Summit	>50	Semi-cured, 20–45 d	7	8	16	0	Medium	Goat and ewe	Raw	3	2	59
37	Summit	1–10	Semi-cured, 20–45 d	8	8	0	0	High	Goat and ewe	Raw	9	2	41
39	Summit	>50	Semi-cured, 20–45 d	11	9	0	0	Medium	Goat and ewe	Raw	1	0	53
3	Moya	1–10	Semi-cured, 20–45 d	8	8	0	0	Minimum	Goat, ewe, and cow	Raw	10	4	38
4	Moya	1–10	Semi-cured, 20–45 d	8	8	0	0	Minimum	Goat, ewe, and cow	Raw	7	4	35
5	Moya	10–50	Semi-cured, 20–45 d	17	17	0	0	Medium	Goat, ewe, and cow	Raw	10	2	63
35	Moya	1–10	Semi-cured, 20–45 d	9	9	0	0	High	Goat, ewe, and cow	Raw	10	2	41
38	Moya	1–10	Semi-cured, 20–45 d	4	4	0	0	High	Goat, ewe, and cow	Raw	12	4	37

[1]Handling: degree of handling during cheese making. Knowledge test: food handlers' hygiene knowledge test. CD test: cleaning and disinfection test. HAS: hygiene assessment system.

[2]This farm only produced soft cheese with raw milk.

Table 2. Summary of the microbiological criteria for cheese and milk by European Union regulations (European Commission, 2004, 2007) and the microbiology quality criteria used in this study (cheese and milk samples)

Sample	Food safety criteria		Process hygiene criteria				Total viable count (cfu/g)
			Staphylococcus aureus (cfu/g)		*Escherichia coli* (cfu/g)		
	Listeria monocytogenes	*Salmonella* spp.	m[1]	M[1]	m	M	
Cheese made from milk that underwent heat treatment	—[2]	—			<100	<1,000	
Cheese made from raw milk	—	—	$<10^4$	$<10^5$			
Cheese made from milk that underwent lower heat treatment than pasteurization	—	—	<100	<1,000			
and ripened cheese made from milk that underwent pasteurization							
Fresh cheese made from milk that underwent pasteurization or stronger heat treatment	—	—	<10	<100			
Cheese samples	—	—		$<10^5/<100^3$		<1,000	
Milk samples	—	—		$<10^5$		<1,000	5×10^5

[1]m: upper microbiological limit on the acceptable concentration in the analytical unit; M: nonconforming analytical units.
[2]Absent in 25 g.
[3]Values in cheese samples made from raw milk/pasteurized milk.

logistic regression models. To determine the risk factors that remained independently associated with each variable of contamination (counts of *E. coli*, *Staph. aureus*, and TVC), multidimensional logistic analyses were carried out. In each analysis, all risk factors associated with the contamination variable in the univariate analysis were introduced. A selection was made by a retrospective method based on Akaike's information criterion. For the resulting model, the adjusted odds ratios of the selected risk factors were obtained and significance was estimated by 95% confidence intervals; statistical significance was set at $P < 0.05$. Data were analyzed with the R package, version 3.1.0 (R Development Core Team, 2014).

RESULTS AND DISCUSSION

Microbiological Results

The results of the microbial counts are summarized in Table 4, which shows the microbiological results that did not comply with the stipulated European standards (European Commission, 2004, 2007).

Results in milk showed that 187 of 469 raw milk samples (40%) did not comply with the stipulated standards for TVC ($<5 \times 10^5$ cfu/mL; European Commission, 2004). The other analyzed microorganism results are shown in Table 4. The high TVC in milk agreed with those indicated in other studies (Smigic et al., 2012; Belli et al., 2013; Walcher et al., 2014).

Our results match those of other authors who have reported the presence of some microorganisms such as *Staph. aureus*, *E. coli*, *Salmonella* spp., and *L. monocytogenes* in raw milk (D'Amico, and Donnelly, 2008). Others obtained higher counts than those detected in our study (Irkin, 2010; Frece et al., 2010; Rysha et al., 2014). Microbiological contamination of milk usually originates from inside the udder (clinical or subclinical mastitis) or from the outer udder side, the surface of equipment and utensils, or from milk storage facilities (Bonfoha et al., 1990). All causes are related to farm management. The milk contamination observed in our studied samples could originate from animal housing or from contaminated goat, cow, or sheep udders, as they were not cleaned before milking; most small farms did not resort to postmilking teat disinfection. Similar sources of contamination have been reported in studies done in Pecorino, Monte Veronese cheese, and Portuguese raw milk cheese by Poli et al. (2007), Giammanco et al. (2011), and Kongo et al., (2008), respectively. In the current study, the high counts in the raw milk samples may have resulted from inadequate storage conditions in milk collection stations (bulk cooling milk tanks).

The microbiological results obtained for the raw and pasteurized milk cheeses are given in Table 4. Only 35 samples (9.1%) had values higher than the stipulated standards (European Commission, 2007). Similar counts have been performed in other studies (Gil et al., 2007; Little et al., 2008; Williams and Withers, 2010; Dambrosio et al. 2013) and associated with foodborne outbreaks caused by the consumption of various cheese types (Haeghebaert et al., 2003; Conedera et al., 2004; Pastore et al., 2008).

Table 3. Incidence of microbiological contamination on milk and cheese production in relation to the potential risk factor considered during cheese production on cheese farms[1]

Factor[2]	Category (no. of dairies)	N_1	*Escherichia coli*, % (95% CI)	*P*-value[3]	*Staphylococcus aureus*, % (95% CI)	*P*-value	N_2	TVC, % (95% CI)	*P*-value
Zone	Coast (15)	309	2.27 (1.1; 4.7)	0.494	2.59 (1.3; 5.1)	0.413	197	9.14 (5.8; 14.0)	0.399
	Midlands (14)	294	2.72 (1.4; 5.3)		3.40 (1.8; 6.2)		166	9.04 (5.5; 14.4)	
	Summit (5)	78	3.85 (1.2; 11.3)		3.85 (1.2; 11.3)		46	8.70 (3.3; 21.0)	
	Moya (5)	92	5.43 (2.3; 12.4)		6.52 (3.0; 13.8)		60	16.67 (9.2; 28.3)	
Production	1–10 kg (8)	122	3.28 (1.2; 8.4)	0.662	5.74 (2.8; 11.5)	0.382	42	21.43 (11.5; 36.3)	0.059
	10–50 kg (14)	312	3.53 (2.0; 6.2)		3.21 (1.7; 5.8)		206	9.71 (6.3; 14.6)	
	>50 kg (17)	339	2.36 (1.2; 4.6)		2.95 (1.6; 5.4)		221	8.14 (5.2; 12.6)	
Type of cheese	Fresh (8)	182	3.85 (1.8; 7.8)	0.066	3.30 (1.5; 7.1)	0.789	134	8.21 (4.6; 14.2)	0.688
	Soft (4)	82	0		4.88 (1.8; 12.3)		34	11.76 (4.5; 27.5)	
	Semi-cured (27)	509	3.14 (1.9; 5.1)		3.34 (2.1; 5.3)		301	10.63 (7.6; 14.6)	
Handling	High (10)	163	2.45 (0.9; 6.3)	0.254	4.91 (2.5; 9.5)	0.357	67	19.40 (11.6; 30.6)	0.017
	Medium (20)	459	3.70 (2.3; 5.9)		3.49 (2.1; 5.6)		337	9.20 (6.5; 12.8)	
	Minimum (9)	151	1.32 (0.3; 5.1)		1.99 (0.6; 6.0)		65	4.62 (1.5; 13.4)	
Knowledge test	<5 (10)	234	0.85 (0.2; 3.3)	0.011	1.71 (0.6; 4.5)	0.058	186	10.22 (6.6; 15.5)	0.910
	≥5 (29)	539	3.90 (2.5; 5.9)		4.27 (2.8; 6.3)		283	9.89 (6.9; 14.0)	
CD test	≤2 (17)	378	3.97 (2.4; 6.5)	0.110	2.91 (1.6; 5.2)	0.386	312	9.94 (7.1; 13.8)	0.931
	>2 (22)	395	2.03 (1.0; 4.0)		4.05 (2.5; 6.5)		157	10.19 (6.3; 16.0)	
HAS	≤45 (8)	122	1.64 (0.4; 6.3)	0.439	4.92 (2.2; 10.5)	0.400	42	21.43 (11.5; 36.3)	0.069
	46–65 (25)	539	2.97 (1.8; 4.8)		3.53 (2.3; 5.5)		395	8.86 (6.4; 12.1)	
	>65 (6)	112	4.46 (1.8; 10.3)		1.79 (0.4; 6.9)		32	9.38 (3.1; 25.3)	
Ewe milk	No (16)	273	4.76 (2.8; 8.0)	0.035	2.93 (1.5; 5.7)	0.524	63	9.52 (4.3; 19.6)	0.887
	Yes (23)	500	2.00 (1.1; 3.7)		3.80 (2.4; 5.9)		406	10.10 (7.5; 13.4)	
Cow milk	No (29)	592	2.70 (1.7; 4.5)	0.433	3.04 (1.9; 4.8)	0.234	384	8.59 (6.2; 11.8)	0.039
	Yes (10)	181	3.87 (1.8; 7.9)		4.97 (2.6; 9.3)		85	16.47 (10.0; 25.9)	

[1]N_1 = number of milk and cheese samples where contamination by *E. coli* and *Staph. aureus* was evaluated according to the level of each factor (773 in all); N_2 = number of cheese samples where contamination was evaluated by total viable count (TVC) (469 in all); *E. coli*, % = percentage of N_1 where contamination by *E. coli* was detected; *Staph. aureus*, % = percentage of N_1 where contamination by *S. aureus* was detected; TVC, % = percentage of N_2 where contamination by TVC was detected.

[2]Handling: degree of handling during cheese making. Knowledge test: food handlers' hygiene knowledge test. CD test: cleaning and disinfection test. HAS: hygiene assessment system.

[3]*P*-value corresponding to the likelihood ratio test.

The *Staph. aureus* counts in contaminated milk were twice that in cheese. Similar results were found by Jakobsen et al. (2011), Gil et al. (2007), Dambrosio et al. (2013), and Hunt et al. (2012) in different analyzed cheeses. Studies into the origin of *Staph. aureus* have indicated that *Staph. aureus* shed from milk or infected udders is the most important contamination source in caprine (Callon et al., 2008) and bovine (Jørgensen et al., 2005) raw milk cheeses. This could also be the case in our study, where all the *Staph. aureus*-positive samples, 10 cheeses, were made from raw milk. The highest *Staph. aureus* contamination levels were reached in both caprine and bovine cheeses sampled at 5 to 6 h after the first pressing (Jakobsen et al., 2011). Other studies have reported that the maximum *Staph. aureus* levels occurred after 24 h in raw bovine (Delbes et al., 2006) and caprine (Vernozy-Rozand et al., 1998) milk cheeses.

The different values observed in our study between the milk and cheese samples for *Staph. aureus* could be due to cheese making not allowing bacteria growth, although the manufacturer can reduce the amount of growth by different measures, such as proper temperature control (4°C) and timely addition of starters (Charlier et al. 2009). During manufacture of semi-hard cheeses, the *Staph. aureus* population is subjected to changes from several stressors, such as a decrease in pH, reduced free water activity (a_w), and competition with starter cultures (Pexara et al. 2012).

The level of contamination by *E. coli* was higher in cheese than in milk. However, no criteria exist for *E. coli* in cheeses made from raw or pasteurized milk in European Commission (2007) regulation.

The levels of contamination of milk and cheese agree with values obtained in other studies performed in different European regions (Zárate et al., 1997; Little et al., 2008; Gil et al., 2007). In the present study, only 2 pasteurized milk samples and 20 raw milk cheese samples showed unsatisfactory microbiological quality for *E. coli*. These results could be due to the conditions under which the Gran Canaria cheeses are processed (facilities, milk storage temperature, cleaning), the physical retention of microorganisms in curds, and partly to microbial growth during coagulation (Diezhandino et al., 2015) and the potential presence of bacteria carriers among staff members.

The cheese samples that showed contamination by *Salmonella* spp. and *L. monocytogenes* were made from raw milk, and these microorganisms were not present in the cheeses made from the same milk.

Determination of Risk Factors for Contamination in Milk and Cheese

Statistical analysis showed the relationship between risk factors and microbiological contamination (*E. coli*, *Staph. aureus*, and TVC) in both cheese and milk. When milk and cheese were studied separately, the statistical results were only descriptive and dubiously inferential. The rare presence of samples contaminated by *Salmonella* spp. and *L. monocytogenes* did not allow us to perform statistical analysis for these microorganisms.

Table 4 shows the results of the microbiological analysis done with the cheese farm products (milk and cheese), which were grouped by the potential risk factors considered during cheese production (Table 3). Values are presented as the number of samples (milk and cheese) contaminated by *Staph. aureus*, *E. coli*, and TVC (in milk only), and the frequency of each in relation to the total of each category. The contamination rate per microorganism was estimated by the 95% CI (Table 3).

Four zones were described for the "zone" factor; Moya was the most contaminated area for all 3 types of microorganisms studied. In relation to the "production" factor, the dairies most contaminated by TVC and *Staph. aureus* were those that made 1 to 10 kg of cheese per day. The dairies with intermediate production rates (10–50 kg/d) had the highest *E. coli* counts. For "type of cheese," soft cheese was the most contaminated by *S. aureus* and TVC, whereas fresh cheese was the most contaminated by *E. coli*. For "handling factor," farms with an intermediate handling level had the highest level of contamination by *E. coli*, whereas those with a high handling level showed the highest level of contamination by *Staph. aureus* and TVC. For the "knowledge" factor, cheese farms with low scores (>5 errors) had high levels of contamination by both *E.*

Table 4. Summary of the microbiological contamination results (number of milk and cheese samples, with % contaminated in parentheses) of the samples of raw milk and cheese made from raw or pasteurized milk

Sample (total no.)	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella*	*Listeria monocytogenes*	Total viable count (TVC)	Total
Milk (387) + (82)[1]	17 (4.39)	2 (0.52)	1 (0.25)	2 (0.50)	187 (40)	212 (45.20)
Cheese (386)	10 (2.6)	20 (5.18)	3 (0.78)	2 (0.52)	—	35 (9.10)
Total (855)	27 (3.50)	22 (2.84)	4 (0.52)	4 (0.52)	187 (40)	244 (28.53)

[1]Only 82 milk samples were analyzed for TVC and 387 for *Staph. aureus, E. coli, Salmonella* spp., *L. monocytogenes*, and TVC.

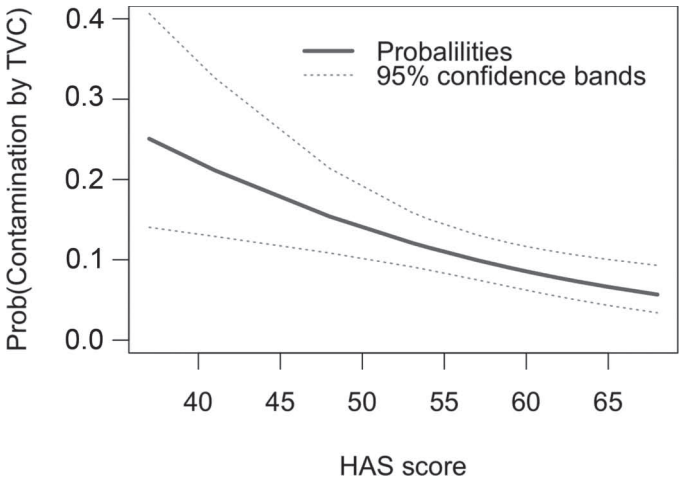

Figure 2. Probability of contamination by total viable count according to hygiene assessment system rating (95% CI). TVC = total viable count; HAS = hygiene assessment system.

coli and *Staph. aureus*. For "CD test," farms with low scores (>2 errors) showed a high level of contamination by *Staph. aureus* and TVC. For the "HAS" factor, farms with <45 points had high counts for *Staph. aureus* and TVC, whereas those with >65 points had higher counts for *E. coli*. The cheese farms that used ewe milk had higher counts for *Staph. aureus* and TVC than for *E. coli*. Those cheese dairies that used cow milk had the highest bacteriological counts for the 3 microorganisms studied. Table 1 shows the score obtained and the errors for each cheese farm in reference to the knowledge test, CD test, and HAS.

In summary, significant differences ($P < 0.05$) were noted in handling factor for TVC, knowledge factor for *E. coli*, and type of milk factor for *E. coli* and TVC. Other factors, such as production and HAS survey, had almost significant (0.059 and 0.069, respectively) *P*-values.

For milk treatment, the greatest number of samples contaminated by *S. aureus* and *E. coli* was found in raw milk, and a difference of more than 2% was obtained between raw and pasteurized milk (3.5 and 1.1%, respectively).

Table 5. Multidimensional logistic model for *Escherichia coli*

Item	*P*-value[1]	Odds ratio (95% CI)
Knowledge test[2] >5	0.039	4.66 (1.08; 20.07)
Ewe milk cheese	0.038	0.41 (0.18; 0.96)

[1]*P*-value corresponding to the likelihood ratio test.

[2]Knowledge test: food handlers' hygiene knowledge test.

The multidimensional logistic analysis for *E. coli* is summarized in Table 5. The factors that showed an independent association with contamination by *E. coli* were those with a score >5 in the knowledge test (odds ratio = 4.66; 95% CI = 1.08–20.07) and those that used ewe milk (odds ratio = 0.41; 95% CI = 0.18–0.96). For *Staph. aureus* contamination, the only variable that showed an association at $P < 0.1$ was that shown in the test factor ($P = 0.058$), which is why we did not perform the multivariate analysis. For TVC, the selection of variables included only the HAS survey score. Figure 2 shows the probabilities of contamination by TVC and the bands that correspond to 95% CI.

CONCLUSIONS

This study attempted to identify the potential microbial risk on cheese farms and how it can be related to different risk factors. Our findings support previous information about these factors to help implement and manage HACCP. This study focused on evaluating dairies but not the facilities of farms and animal handling during milking because contamination of milk and cheese, especially with *Staph. aureus*, is due to possible deficiencies on farms. Studies on risk factors should be extended to potential risk factors that may occur when obtaining and maintaining milk until its processing, especially during milking. Despite the limitations of this study, we were able to elucidate clear relationships between microbiological risk and risk factors, which could offer benefits to food safety programs on Gran Canaria cheese farms. We believe that these results can be extrapolated to cheese-making areas elsewhere in the world.

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