Research & Reviews: Journal of Food and Dairy Technology

e-ISSN:2321-6204
p-ISSN:2347-2359

Behavior Of *Listeria* Monocytogenes in the Sheep Raw Milk Cheese: A Study at Different Stages Of Production and Shelf Life

N'Guessan Elise[1*], Amara Nassima[1], Soro Doudjo[2], Godrie Therese[1] and Sindic Marianne[1]

[1]Analysis, Quality and Risk Unit, Laboratory of Agro-food Quality and Safety, Gembloux Agro-Bio Tech, University of Liege, Passage des Déportés, 2, BE-5030 Gembloux, Belgium

[2]Food and Science Department, Institut National polytechnic Felix Houphouet-Bougny Yamoussoukro, Ivory Coast

Research Article

Received Date: 24/03/2017
Accepted Date: 31/03/2017
Published Date: 07/04/2017

***For Correspondence**

N'Guessan Elise, Analysis, Quality and Risk Unit, Laboratory of Agro-food Quality and Safety, Gembloux Agro-Bio Tech, University of Liege, Passage des Déportés, 2, BE-5030 Gembloux, Belgium Tel: +32 498159748.

E-mail: enguessan@yahoo.fr

Keywords: Raw milk cheeses, Soft cheese, *Listeria* monocytogenes, Behaviour, Farm

ABSTRACT

Cheese is a ready-to-eat food easily contaminated on the surface by undesirable microorganisms. Even if some are spoilage microorganisms, others are pathogenic such as *L. monocytogenes*, which have been associated with foodborne listeriosis by consumption of cheese. Here, we investigated through aging tests, the behaviour of *L. monocytogenes* during soft and pressed cheeses ripening and during their shelf-life. The contamination level relative to *L. monocytogenes* of 18 samples including fresh, soft and pressed cheeses was first assessed through microbiological analysis. Then soft and pressed cheeses manufactured from sheep raw milk, intentionally contaminated by *L. monocytogenes* were sampled in order to follow this pathogen behaviour during the ripening and shelf-life. The ripening period resulted in an increasing in pH and water activity. Likewise, a growth of *L. monocytogenes* of 0.7 log (ufc/g) and 0.6 log (ufc/g) were observed for the soft and pressed cheeses respectively. Furthermore, the storage at 4°C led to the decrease of the *L. monocytogenes* population without causing the total disappearance of this pathogen. Therefore, adequate hygienic practices are needed at every stage of the process to control the growth of the bacteria.

INTRODUCTION

Listeria monocytogenes is an important gram-positive food-borne pathogen. This bacterium is able to cause listeriosis in animals and in humans populations [1]. Foodborne listeriosis is relatively rare but is a serious disease with high fatality rates (20%–30%) compared with other food-borne microbial pathogens [2,3]. *L. monocytogenes* is included in the safety criteria regulation (EC) No 2073/2005 for dairy products because of its ability to contaminate dairy products. The significance of *L.monocytogenes* in cheese and in samples from dairy plants has been previously reported [4-6]. This bacterium is a ubiquitous pathogen with many possible modes of entry into dairy processing facilities. Furthermore, Parisi et al. [7] highlighted the wide-spread presence of *L. monocytogenes* in cheese factories. According to that study, this bacterium can persist for long periods of time, resulting in a continuous contamination of the dairy products. This is of concern because *L. monocytogenes* has been shown to cause life-threatening disease in foetuses, newborns, immune compromised people and the elderly [8]. Moreover, after the isolation of strains with similar profile from different sampling sites, within and among cheese making plants, Spanu et al. [9] suggested a possible transfer of *L. monocytogenes* contamination along production lines and from one facility to another. The survival and growth of *L. monocytogenes* in a dairy environment depends on the manufacturing, ripening and storage conditions used for the cheeses, even when the cheese is stored at refrigeration temperatures [10,11]. Unlike many food borne pathogens, *L. monocytogenes* can survive and grow over a wide range of environmental conditions such as refrigeration temperatures, low pH and high salt concentration. This allows the pathogen to overcome food preservation and safety barriers, and pose potential risk to human health. Analytical data generated for several years show that low levels, <100 cfu/g of *L. monocytogenes* contamination are observed in raw milk cheeses [12]. When the behaviour of the pathogen in a product under particular conditions is unknown, studies to gather the experimental data concerning the implicated product are recommended [11,13]. *L. monocytogenes* has been indicated

e-ISSN:2321-6204
p-ISSN:2347-2359

as one of the principal pathogens of concern associated with dairy products, with particular reference to raw unpasteurized milk and its derivative products. Literature reports several food borne diseases involving *L. monocytogenes* and cheeses in different countries. In 2005, ten cases of listeriosis in a small area of Switzerland were due to locally made and distributed soft cheese; when, in 2006, the Czech Republic experienced one large outbreak, involving 78 patients, of whom 13 died here also, soft cheese was identified as the source. Likewise, a US multistate outbreak of listeriosis was linked to ricotta salata imported from Italia, in 2012. Concerning this incident, the cheese that cause the outbreak was produced in a plant in Apulia that processed semi-finished cheeses supplied by five plants in Sardinia [2,14-17]. Furthermore, according to the U.S. Food and Drug Administration Food Code, most cheeses are potentially hazardous foods based on pH and water activity [18]. Nevertheless, the consumption of raw unpasteurized milk and raw milk cheeses all the word is greatly increasing [19-24]. The study of *L. monocytogenes* behaviour in cheeses made from raw sheep's milk could help understand if and how this pathogen is likely to grow in these dairy products. Thus, to prevent food poisoning and avoid potential risk to human health, it is essential to identify the process steps that might be favourable to the development of *L. monocytogenes*, and to implement correctives actions. Here, we followed through aging tests, *L. monocytogenes* behaviour during cheeses ripening and during their shelf-life, in order to ensure that an initial contamination of less than 100 cfu/g at the end of the production would not exceeded the limit (≤ 100 cfu/g for food products) recommended by the European regulation at the end of shelf-life.

MATERIALS AND METHODS

Cheese Level Contamination

Dairy products including sheep raw milk, soft, fresh and pressed cheeses from a Belgian dairy plant were used in this work. The contamination level of these products, relative to *L. monocytogenes* was first assessed through physicochemical and microbiological analyses, performed according to the NF EN ISO 11290-2/A1 (02/2005). The purpose was to select a type of cheese favourable for aging study.

Microbiological Analysis

Microbiological analyses were carried out as per the standard method (ISO, 1998). For this, 25 g /25 ml of cheese or raw milk, were weighed in sterile stomacher bags 400 (Led Techno) and mixed with 225 ml of sterile peptone buffer water. The samples were then dispersed by stomaching for 1 min at 230 rpm in a stomacher 400. A tenfold serial dilution was made, and from the appropriate dilution, 100 µl was spread plated onto baird barker, sabaureud or depth inoculation for the plate count agar (PCA), Violet Red Bile Lactose Agar (VRBL), Man Rogosa and Sharpe Agar (MRS). Following by the incubation at different temperatures and times, depending on the culture media and the microorganisms sought. The bacteria enumeration was performed according to the existing standards.

Physicochemical Analysis

The principle of pH measurement is based on the difference in chemical potential existing between ions electrode glass and reference electrode (calomel-kcl) immersed in the same solution, when the water activity was measured by the dew point chilled mirror technique.

Raw Milk and Cheeses Analysis

In order to gain a precise idea of the raw milk contamination level, fifteen samples of sheep raw milk were analysed for the detection of *L. monocytogenes*, according to the standard NF EB ISO 11290-2/A1 (02/2005). Raw milk intentionally contaminated with *L. monocytogenes* was thereafter used for the soft and pressed cheeses production. These cheeses were then sampled to monitor the behavior of *L. monocytogenes* during maturation and shelf life, either a follow-up on the entire life cycle. In addition, lactic bacteria and mesophilic aerobic bacteria were enumerated.

RESULTS AND DISCUSSION

The regulation of *L. monocytogenes* behavior in raw milk cheese could help in food poisoning prevention and to avoid potential risk to human health. As illustrated in the present study, the follow of this pathogen in cheeses reminds essential. Here, we investigated the behaviour of *L. monocytogenes* during soft and pressed cheeses ripening and during their shelf-life through aging tests. **Table 1** summarize the results related to the physico-chemical characteristics and the contamination level of different dairy products including fresh, soft and pressed cheeses before *L monocytogenes* inoculation. The pH values <4.5 were observed in fresh cheese samples analyzed and *L. monocytogenes* grew in only two out of twelve cheese samples. The growth of this pathogen was ranged from 3.7 to 4 log cfu/g. The low pH, survival and growth of *L. monocytogenes,* whose minimum growth pH is around 4.3 [3,25,26]. By contrast, the enzymatic technology of soft and pressed cheeses contributes to increasing the pH ranged from 4.5 to 4.8 and 4.8 to 5.2 respectively (24 h after salting). Soft cheese with higher moisture content (0.97-0.99) may have provided a more favourable environment for microbial growth **(Table 2)** [27,28]. On the basis of these results, the soft and pressed cheeses were selected to study the behaviour of *L. monocytogenes* in cheeses, during their ripening and shelf-life.

e-ISSN:2321-6204
p-ISSN:2347-2359

Table 1. Physico-chemical and microbiological characteristics of fresh-cheeses.

Products	Ages (days)	Samples numbers	Physicochemical parameters		*L. monocytogene* (log ufc/g)	Mesophilic aerobic bacteria (log ufc/g)	Lactic bacteria (log ufc/g)
			pH	aw			
Fresh cheese	15	1	4.36 ± 0.02	0.982 ± 0.001	Abs	9.0	9.0
					3.7	9.0	6.3
					4.0	9.0	6.0
	20	5	4.30 ± 0.04	0.997 ± 0.001	Abs	9.0	7.0
					Abs	9.0	7.0
					Abs	9.0	7.0
					Abs	9,0	7.0
					Abs	9.0	7.0
Fresh cheese «crottin frais»	24	5	4.48 ± 0.08	0.998 ± 0.001	Abs	9.0	5.8
					Abs	9.0	7.0
					Abs	9.0	5.8
	25	1	4.46 ± 0.05	0.963 ± 0.006	Abs	9.0	8.6

Table 2. Physico-chemical and microbiological characteristics of soft and pressed cheeses.

Products	Ages (days)	Samples numbers	Physico-chemical parameters		*L. monocytogenes* (log ufc/g)	Mesophilic aerobic bacteria (log ufc/g)	Lactic bacteria (log ufc/g)
			pH	a_w			
Soft cheese	42	5	4.66 ± 0,02	0.982 ± 0.015	Abs	9.0	7.0
					Abs	9.0	7.5
					2.5	9.0	6.3
					Abs	9.0	5.9
					Abs	9.0	8.7
Pressed cheese	25	1	4.46 ± 0.05	0.963 ± 0.006	4	9.0	8.7

The Contamination Rate of *L. Monocytogenes* in Sheep Raw Milk

The contamination level of the raw milk used for the soft and pressed cheeses production was determined before its inoculation by the *L. monocytogenes* strain. According to the results presented in **Table 3**, *L. monocytogenes* was detected in two out of fifteen milk samples analyzed. Previously recognized sources of raw milk contamination by *L. monocytogenes* have been highlighted by different authors [29-31]. According to these studies, the raw milk contamination could be linked the environment, feed, cattle feces, wild life, biofilm on the milking machine or improper milking procedures. Mesophilic aerobic bacteria were isolated from all raw milk samples tested **(Table 3)**. Applying the criteria in Recommendation EC 2073/2005, these samples were above the legal limits for the mesophilic aerobic bacteria. This high level of these bacteria may lead to an improper milking procedure. Similarly, Allonso-Calleja et al. [32] have isolated high (7.66 log cfu/g) counts of aerobic mesophilic bacteria from a raw goat's milk cheese during the manufacturing and ripening processes. The increasing bacteria in raw milk sampled from dairy farms could be linked to the temperature or time, as observed by Vithanage et al. [33]. Furthermore, survey from various countries have monitored the presence of different types of pathogens in raw milk, with prevalence levels as high as 13% for bacteria like *Campylobacter jejuni* and *L. monocytogenes* [34]. The relative importance of the various sources of contamination not only depends on the farming practices, but may also be different for each pathogen, according to Soboleva T [35].

Table 3. Physicochemical and microbiologycal analysis of sheep raw milk analyses.

N° Samples	Physicochemical parameters		Bacteria	
	pH	a_w	Mesophilic aerobic bacteria log (ufc/ml)	*L. monocytogenes* log (cfu/ml)
1	6.56 ± 0.02	0.999 ± 0.012	10	Abs
2	6.66 ± 0.13	1.010 ± 0.023	9	Abs
3	6.63 ± 0.05	0.990 ± 0.001	9	1.8
4	6.68 ± 0.08	0.990 ± 0.009	9	Abs
5	6.69 ± 0.02	0.990 ± 0.005	9	Abs

e-ISSN:2321-6204
p-ISSN:2347-2359

6	6.69 ± 0.02	1.000 ± 0.001	9	Abs
7	6.72 ± 0.06	1.001 ± 0.007	9	Abs
8	6.75 ± 0.03	1.000 ± 0.001	10	1.5
9	6.75 ± 0.01	0.999 ± 0.005	10	Abs
10	6.65 ± 0.01	0.999 ± 0.007	9	Abs
11	6.85 ± 0.01	1.000 ± 0.019	9	Abs
12	6.53 ± 0.04	1.001 ± 0.001	7	Abs
13	6.63 ± 0.09	0.999 ± 0.007	8	Abs
14	6.70 ± 0.02	0.999 ± 0.006	7	Abs
15	6.42 ± 0.01	0.999 ± 0.004	9.6	Abs

The Behavior of *L. monocytogenes* in Soft and Pressed Cheeses during the Ripening and Shelf-life

The evolution of *L. monocytogenes* counts has been studied in soft and pressed cheeses manufactured from sheep raw milk, intentionally contaminated by this pathogen. The monitoring was carried up for the ripening period and cold storage at 4°C. An increasing of *L. monocytogenes* number in soft cheese was observed during the ripening, ranging from 4.2 ± 0.1 log cfu/g to 4.9 ± 0.5 log cfu/g at the end of the ripening period, meaning an increase of 0.7 log cfu/g. This growth could be related to the favorable conditions including pH increasing at the beginning of ripening time **(Table 4)**, that is in line with previous studies [28,36,37]. Witch showed that favorable properties include pH and $a_{w,}$ for growth initiation at the early stages of the cheese making process, could lead to high development of *L. monocytogenes* populations. Furthermore, most cheeses are potentially hazardous foods based on pH and water activity, as observed the U.S Food and Drug Administration Food Code [18]. However, a low variability in *L. monocytogenes* growth in raw milk cheeses during the ripening step has been highlighted. During the cold storage (4°C), the cessation of *L. monocytogenes* growth in soft cheeses was observed. The population decreased from 4.9 ± 0.5 to 4.4 ± 0.2 log cfu/g, following by a stationary phase with *L. monocytogenes* concentration maintained about 4.4 ± 0.2 log cfu/g until the cheese shelf-life end **(Table 4)**. The inhibition of *L. monocytogenes* growth by the amount of organic acid produced by the lactic bacteria can explain these results. Furthermore, the decrease in water activity during the stationary phase did not result in the disappearance of the pathogen. *L. monocytogenes* has survived in cheese but not multiply. It was observed a similar *L. monocytogenes* growth in a cheese with a flowered crust like Camembert. **Table 4** also shows a stability of the mesophilic aerobic bacteria and lactic bacteria counts during the ripening phase, unlike lactic bacteria rate, which decreased from 8.1 ± 0.1 log cfu/g to 6.6 ± 0.4 log cfu/g, during the stationary step. Concerning the pressed cheese, similar results were observed, with an increasing of *L. monocytogenes* count from 3.3 ± 0.3 log cfu/g to 3.9 ± 0.3 log cfu/g at the end of the ripening. During the storage, the pH and water activity conditions have given rise to a reduction of *L. monocytogenes* rate **(Table 5)**. In the same vein, Chatelard- Chauvin et al. [38] observed a significant decrease of *L. monocytogenes* in the uncooked pressed cheese rinds after 45 days of storage. According to them, the low a_w would be responsible for this decrease.

Table 4. Evolution of physico-chemical and microbiological characteristics of soft cheese during ripening and shelf-life.

Steps	Days	pH	a_w	*L. monocytogenes*	Total count	Lactic bacteria
Ripening (12°C/95% HR)	J+24	5.64 ± 0.18	0.983 ± 0.000	4.2 ± 0.1	8.4 ± 0.5	8.1 ± 0.1
	J+30	5.90 ± 0.29	0.993 ± 0.005	4.4 ± 0.1	8.1 ± 0.5	7.2 ± 0.1
	J+37	6.95 ± 0.66	0.995 ± 0.001	4.9 ± 0.5	8.6 ± 0.5	7.5 ± 0.5
	J+44	7.34 ± 0.19	0.994 ± 0.002	4.5 ± 0.1	8.1 ± 0.5	7.1 ± 0.3
Storage at 4°C	J+51	7.32 ± 0.15	0.971 ± 0.001	4.4 ± 0.1	8.1 ± 0.5	6.8 ± 0.1
	J+58	759 ± 0.23	0.968 ± 0.001	4.4 ± 0.1	8.2 ± 0.5	6.6 ± 0.4
	J+65	7.61 ± 0.23	0.968 ± 0.001	4.4 ± 0.2	8.2 ± 0.5	6.6 ± 0.4

Table 5. Evolution of physico-chemical characteristics of pressed cheese during ripening and shelf-life.

Steps	Days	pH	aw	*L. monocytogenes*	Total count	Lactic bacteria
Ripening (12°C/95%HR)	J+30	5.69 ± 0.01	0.98 ± 0.002	3.3 ± 0.3	9.3 ± 0.2	8.6 ± 0.2
	J+37	5.75 ± 0.35	0.98 ± 0.002	3.4 ± 0.2	8.6 ± 0.5	7.5 ± 0.5
	J+44	5.75 ± 0.35	0.98 ± 0.002	3.5 ± 0.6	8.8 ± 0.1	7.5 ± 0.4
	J+51	6.04 ± 0.21	0.983 ± 0.001	3.8 ± 0.0	8.8 ± 0.5	7.4 ± 0.8
	J+58	6.28 ±0.008	0.986 ± 0.003	3.9 ± 0.3	8.5 ± 0.3	7.2 ± 0.5

e-ISSN:2321-6204
p-ISSN:2347-2359

Storage at 4°C	J+65	6.33 ±0.007	0.978 ± 0.008	3.0 ± 0.2	8.0 ± 0.3	6.8 ± 0.4
	J+72	6.1 ± 0.007	0.966 ± 0.002	2.9 ± 0.2	8.1 ± 0.5	6.8 ± 0.5
	J+79	6.12 ±0.21	0.965 ± 0.001	2.8 ± 0.5	8.9 ± 0.1	7.5 ± 0.2
	J+86	6.1 ± 0.02	0.965 ± 0.001	2.6 ± 0.4	7.9 ± 0.3	7.2 ± 0.5
	J+90	6.13 ± 0.01	0.964 ± 0.001	2.5 ± 0.5	7.9 ± 0.3	6.4 ± 0.2

CONCLUSION

Soft and pressed cheeses were used for the study of *L. monocytogenes* behaviour in these foods during their ripening and shelf-life. The ripening period result in an increasing in pH and water activity. Likewise, a growth of *L. monocytogenes* of 0.7 log (ufc/g) and 0.6 log (ufc/g) were observed for the soft and pressed cheeses respectively. However, the storage at cold temperature leads to the decrease of the *L. monocytogenes* population without causing the total disappearance of this pathogen. To avoid any health hazards in these artisanal food products, adequate hygienic practices are needed at every stage of the process to assess and control bacteria growth.

ACKNOWLEDGEMENT

This work was supported by DiversiFerm, a grant from the Public Service of Wallonia, Directorate-General for Agriculture and a grant from the Belgian Development Cooperation.

REFERENCES

1. Farber JM and Peterkin PI. *Listeria monocytogenes*, a food-borne pathogen. Microbiol Rev. 1991;55:476-511.
2. Allerberger F and Wagner M. Listeriosis: a resurgent foodborne infection. Clin microbial infect. 2010;16:16-23.
3. Reda WW, et al. *Listeria monocytogenes*: An emerging food-borne pathogen and its public health implications. J infect dev ctries. 2016;10:149-154.
4. Makino SI, et al. An outbreak of food-borne listeriosis due to cheese in Japan, during 2001. Int J Food Microbiol. 2005;104:189-196.
5. Harakeh S, et al. Antimicrobial resistance of *Listeria monocytogenes* isolated from dairy-based food products. Science of the Total Environment. 2009;407:4022-4027.
6. Cagri-Mehmetoglu A, et al. Incidence of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in two Kasar cheese processing environments. Food Control. 2013;22:762-766.
7. Parisi A, et al. Occurrence of *Listeria* spp. in dairy plants in southern Italy and molecular subtyping of isolates using AFLP. Food Control. *2013;*29:91-97.
8. Schuchat A, et al. Outbreak of neonatal listeriosis association with mineral oil. J Pediatric Infect Dis. 1991;10:183-189.
9. Spanu C, et al. Microbiological challenge testing for *Listeria monocytogenes* in ready-to-eat food: a practical approach. Ital J Food Saf. 2014;3:4518.
10. Pintado CMBS, et al. Control of pathogenic and spoilage microorganisms from cheese surface by whey protein films containing malic acid, nisin and natamycin. Food Control. 2010;21:240-246.
11. Bernini V, et al. The presence, genetic diversity and behavior of *Listeria monocytogenes* in blue-veined cheese rinds during the shelf life. Food control. 2013;34:323-330.
12. Sanchez A, et al. Microbiological analyses of raw milk cheeses from Walloon farms. 15th conference of food microbiology. 2010.
13. Codex Alimentarius. Report of the thirty – fourth session of the codex committee on food hygiene (ALINORM 03/13). Rome, Italy: FAO/WHO. 2002.
14. Vit M, et al. Outbreak of listeriosis in the Czech Republic, late 2006: preliminary report. Euro Surveillance. 2007;12:3132.
15. Bille J, et al. Outbreak of human listeriosis associated with tomme cheese in northwest Switzerland, 2005. Euro Surveillance. 2006;11:91-93.
16. Magalhaes R, et al. Cheese related listeriosis outbreak, Portugal, March 2009 to february 2012. Euro Surveillance. 2015;20:21104.
17. Acciari VA, et al. Tracing sources of Listeria contamination in traditional Italian cheese associated with a US outbreak: investigations in Italy. Epidemiol Infect. 2016;144:2719-19.

e-ISSN:2321-6204
p-ISSN:2347-2359

18. Leong WM, et al. Growth of *Listeria monocytogenes, Salmonella Escherichia coli* O157:H7, and *Staphylococcus aureus* on cheese during extended storage at 25°C. J Food Prot. 2014;77:1275-88.

19. European Commission. Commission Regulation (EC) No/2005 of 15 November 2005 on microbiological criteria for foodstuffs. (L-338). 2005;1-26.

20. Food and Drug Administration. Annex 3 e public health reasons/administrative guidelines. In Food Code U.S (pp. 315e499). College Park, MD 20740: Department of Health and Human Services, Public Health Service, Food and Drug Administration. 2009.

21. Food Standards Australia New Zealand. Proposal P1007. Primary production and processing requirements for raw milk products, 2nd Assessment report. Forsythe, S. J. 2002. The microbiological risk assessment of food. Oxford, United Kingdom: Blackwell Publishing. 2011.

22. National Agency for Veterinary Medicinal Products-french Agency for Food Environmental and occupational Health Safety (ANSES). *Listeria monocytogenes*. 2011.

23. World Health Organization/Food and Agriculture Organization of the United Nations. Risk assessment of *Listeria monocytogenes* in ready-to-eat foods. *Microbial risk assessment*. Series N°4, P.13. 2004.

24. Quero GM, et al. Quantitative detection of *Listeria monocytogenes* in raw milk and soft cheeses: culture-independent versus liquid and solid- based culture-dependent real time PCR approaches. LWT - Food Science. Technology. 2014;28:11-20.

25. Millet L, et al. Control of *Listeria monocytogenes* in raw-milk cheeses. Int J Food Microbiol. 2006;108:105-114.

26. Leistner LL, et al. Basic aspects of food preservation by hurdle technology. Int J Food Microbiol. 2000;55:181-186.

27. Callon C, et al. Ripening conditions : A tool for the control of Listeria monocytogenes in uncooked pressed type cheese. Food Control. 2011;22:1911-1919.

28. Schvartzman MS, et al. Effect of pH and water activity on the growth limits of Listeria monocytogenes in a cheese matrix at two contamination levels. J Food Prot. 2011;74:1805-13.

29. Yoshida T, et al. Sources and routes of contamination of raw milk with Listeria monocytogenes and its control. J Vet Med Sci. 1998;60:1165-1168.

30. Vilar MJ, et al. Prevalence of and risk factors for Listeria monocytogenes species on dairy farms. J dairy sci. 2007;90:5083-8.

31. Latorre AA, et al. Molecular ecology of Listeria monocytogenes: evidence for reservoir in milking equipment on a dairy farm. Appl and environ microbial. 2008;75:1315-1323.

32. Allonso-Calleja C, et al. Changes in the Microflora of Valdeteja Raw Goat's Milk Cheese throughout Manufacturing and Ripening. Lebensm-Wiss. U-technol. 2002;35:222-232.

33. Vithanage NR, et al. Microbiological quality of raw milk attributable to prolonged refrigeration conditions. J dairy res. 2017;84:92-101.

34. John AL. Raw milk comsumption: Risks and Benefits. Nutr Today. 2015;50:189-193.

35. Soboleva T. Assessment of the microbiolocal risks associated with the consumption of raw milk. MPI Technical Paper N° 2014/12. 2014.

36. Beaufort A, et al. Prevalence and growth of *Listeria monocytogenes* in naturally contaminated cold-smoked salmon. Letter Appl Microbiol. 2007;44:406-411.

37. Coroneo V, et al. Detection of virulence genes and growth potential in *Listeria monocytogenes* strains isolated from ricotta salata cheese. J Food Sci. 2016;81:M114-20.

38. Chatelard-Chauvin C, et al. Behavior of *Listeria monocytogenes* in raw milk Cantal type cheeses during making, ripening and storage in different packaging conditions. Food control. 2015;54:53-65.