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Presence of *Listeria monocytogenes* in Chilean food matrices

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ABSTRACT

Objectives: To compare *Listeria monocytogenes* strains obtained from food matrices with those from environmental samples in the same food processing plant.
Methods and results: Between 2008 and 2012 the presence of *L. monocytogenes* was evaluated in 2647 food samples. A total of 448 work surfaces and 92 equipment's were also evaluated from 6 plants which produce ready-to-eat (RTE) foods in Santiago, Chile. An additional selected sample of hand and nails samples was also obtained from 13 food handlers working in a sausage elaboration plant.
As a whole *L. monocytogenes* was present in 265 (10%) food samples and 22 (4%) environmental samples. The foods with highest recovery were red meats 14/60 (23%), poultry 223/1196 (19%), the remaining samples accounted a total of 27/1391 (2%). The environmental samples positive for *L. monocytogenes* were obtained from two food plants both the cheese 8/8 (100%) and from a fresh peaches exporter 3/3 (100%). Finally *L. monocytogenes* was isolated from 5/13 (38%) food handlers studied.
Conclusions: The study confirms the presence of *L. monocytogenes* in different matrices, especially in meat and RTE products. Analyses conducted on work surfaces revealed that contamination comes mostly from both raw materials and surfaces in indirect contact with foods.
Significance and impact of study: The study reinforces the need for companies to apply regulations related to food quality and safety systems (HACCP, Hazard Analysis & Critical Control Points) to prevent *L. monocytogenes* contamination from food processing plants.
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1. Introduction

Listeria monocytogenes is a human and animal foodborne pathogen, which causes listeriosis. Listeriosis is a serious illness with low incidence, but with mortality rate that fluctuates between 20 and 30% of those affected (Cartwright et al., 2013). In the U.S., *L. monocytogenes* is implicated in 2500 cases of listeriosis and approximately 500 deaths annually. For this reason, listeriosis is considered one of the infectious diseases with the most social and economic relevance (Cartwright et al., 2013; Mead et al., 2006).

L. monocytogenes is a ubiquitous bacteria usually found in the environment. The pathogen has been found various food matrices, such as ready-to-eat (RTE) foods, milk and cheeses, cold-cut meats, smoked fish, seafood, and vegetables (EFSA, 2013; Gottlieb et al., 2006; Kakiomenou, Tassou, & Nychas, 1998; Silk et al., 2012). RTE foods have been implicated in most of the major listeriosis outbreaks in the last 30 years (EFSA, 2013; Gottlieb et al., 2006; Kakiomenou et al., 1998; Silk et al., 2012).

The presence of *L. monocytogenes* in food processing plants is a complicated issue due to the psychrotrophic nature of *Listeria* that enables the pathogen to multiply and sustain its presence in biofilms in the environment and machineries and different work surfaces in direct contact with foods (Stepanovic, Cirkovic, Raning, & Svabic-Vlalhovic, 2004).

The most relevant characteristic of *Listeria* is that the presence of multiple virulence mechanisms, for example the ability to survive limits of pH 4.39–9.4 and water activity highest a 0.92 (Chen, Wu, Zhang, Yan, & Wang, 2014; USFDA, 2013; Wałecka-Zacharska, Kosek-Paszkowska, Bania, Karpıskova, & Stefaniak, 2012), its resistance to many disinfectants and notably, its potential to multiply in low temperatures (Vázquez-Boland et al., 2001). These multiple mechanisms make *L. monocytogenes* a priority pathogen that should be included in the hazard analysis and critical control points (HACCP) line of production as well as surveillance and infectious disease control programs (Chao et al., 2006; Green & Kane, 2014).

Cases of listeriosis in humans can appear as outbreaks or as sporadic cases. *L. monocytogenes* is fundamentally an opportunistic pathogen that can severely affect the most vulnerable hosts; pregnant women, newborns, the elderly and patients with compromised immune system (Cartwright et al., 2013; EPI, 2012).

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Listeriosis is frequently caused by contaminated ready-to-eat (RTE) foods, Deli meats and cheese (Chen et al., 2014). Outbreaks have been also reported due to cabbage, unpasteurized milk, fresh cheese, and various raw vegetables (Chao et al., 2006). Listeriosis cases have also been associated due to ingestion of insufficiently cooked poultry (Cartwright et al., 2013; Chiarini, Tyler, Farber, Pagottoy, & Destro, 2009). The presence of *L. monocytogenes* in raw materials (e.g. chicken), in the environment, equipment's, and utensils, as well as in final products has been already reported (Chao et al., 2006; Chiarini et al., 2009).

In Chile, the presence of *L. monocytogenes* in foods is increasing after a large outbreak of listeriosis in 2008 (Foerster, Gonzalez-Hein, Troncoso, & Figueroa, 2012). The outbreak was traced back to consumption of goat cheese and caused 14 deaths. In this outbreak it was possible to confirm through Pulsed Field Gel Electrophoresis (PFGE), the correlation between the presence of the agent in persons who got sick or who died and the isolated pathogen in contaminated foods. After this outbreak in 2008 *L. monocytogenes* was included in the Chilean Food Safety Regulation (*Reglamento Sanitario de los Alimentos*).

The aim of this study was to assess and compare *L. monocytogenes* strains isolated from various food matrices, surfaces and equipment's from establishments previously affected by confirmed outbreaks.

2. Materials and methods

2.1. Experimental design

The presence of *L. monocytogenes* was studied in 11 food matrices (Table 1) which came from cafeterias and food processing plants during 2008–2012. Additionally, a total of 540 environmental samples (448 work surfaces and 92 equipment's) obtained from 6 plants which specialized in production of RTE foods were analyzed.

The food matrices samples were obtained from finished products, the environmental samples were taken during processing and the samples from food handlers were taken during processing.

2.2. Isolation and identification of L. monocytogenes in foods

Microbiological analysis was conducted according to the recommendations in the BAM: Detection and Enumeration of *L. monocytogenes* (USFDA, 2011), except for poultry carcasses, which were processed with according to the methodology established by the Food Safety and Inspection Services (FSIS).

The One-step *Listeria* enrichment was performed in BLEB broth, incubated for 24–48 h at 30 °C. Isolation of *L. monocytogenes* was conducted in Palcam and Aloa chromogenic agar (OXOID, England). Plates were incubated for 24 and 48 h at 37 °C.

Table 1
Presence of *L. monocytogenes* in food matrices and environmental samples (work surfaces and equipment's) sampled between 2008 and 2012.

Plant	Final products			Work surface/Equipment		
	N_t	$N(+)$	%	N_t	$N(+)$	%
A: Cheese	7	7	100	6	3	50
B: Cheese*	8	8	100	25	1	4
C: Peaches	1	1	100	9	2	22
D: Icecream*	108	3	3	102	13	13
E: Cured meat	31	2	6	5	0	0
F: Seafood	179	5	3	393	3	1

The asterisk means that both foods (cheese and icecream) were associated to outbreaks.

Presumptive *L. monocytogenes* colonies were tested through Gram stain and mobility at 25 °C, catalase, and CAMP tests (USFDA, 2011).

DNA from biochemically suspicious strains were extracted and confirmed through PCR. Briefly, the PCR primers used to amplifying the *iap* gene of *L. monocytogenes* were MonoA (5′- AAACTGCTAAC ACAGCTACT-3′) and MonoB (5′ GCACTTGAATTGCTGTTATTG-3′). The expected size of the *iap* PCR product is 348 bp as described by Bubert et al. (1999), Hoorfar et al. (2004), Malorny et al. (2003). PCR was performed using templates from boiled lysates of 10^6 cells of *L. monocytogenes*. The PCR contained 6.5 μL GoTaq Green Master Mix (Promega), 1.3 μL of each primer (1 μmol), 0.6 μL DNA template and water to give a final reaction volume of 13 μL according to standard protocols (Bubert et al., 1999; Hoorfar et al., 2004; Malorny et al., 2003).

Amplifications started with an initial denaturation step at 94 °C for 3 min, followed by 30 cycles, each at 94 °C for 1 min, 58 °C for 45 s, and 72 °C for 45 s in (MiniCycler, MJ Research, Canada). PCR-amplified products were visualized in 1% agarose gels in 1× Tris-acetate–EDTA (pH 7.8) buffer with ethidium bromide (0.2 μg/ml) by using a UV transilluminator (VL-TFP-M/WL, Viber Lourmat, France).

2.3. Detection of L. monocytogenes in work surfaces and equipment's

Environmental samples were obtained from 6 food establishments selected because of their association with previous *L. monocytogenes* outbreaks. These included an artisanal (Plant A) and one industrial (Plant B) cheese producer, a canned peaches plant (Plant C), one ice-cream producer (Plant D), one sausage plant (Plant E) and finally a seafood processor (Plant F). The surface samples were obtained during processing by swabbing a 10 × 10 cm area using a sterile swab, which was then transferred to a tube containing 5 ml of *Listeria* enrichment broth. Samples were then incubated for 24–48 h at 30 °C. *L. monocytogenes* isolation was conducted with Palcam and Aloa plate agar and the positive colonies were confirmed by means of PCR as previously mentioned.

2.4. Search of L. monocytogenes in food handlers

The hand and nail samples obtained from food handlers were obtained from Plant E. Food handlers washed their hands during processing in a sterile bag with 25 ml of the transport medium (Cary y Blair, BBL), bags were then centrifuged at 5000 rpm for 15 min, supernatants was removed and the pellet suspended in 2 ml of sterile saline. Nail samples were taken with a sterile toothpick. Subsequent enrichment was performed on both samples in a *L. monocytogenes* broth incubated for 24–48 h at 30 °C, the inoculum were then transferred to selective two agar plates (Aloa and Palcam) and suspicious colonies for were confirmedas *L. monocytogenes* by PCR using the methodology described previously. (Bubert et al., 1999; Hoorfar et al., 2004; Malorny et al., 2003).

2.5. L. monocytogenes characterization through Pulsed-Field Gel Electrophoresis (PFGE)

In order to determine the possible contamination origin of *L. monocytogenes*, analysis of final products as well Plant environment and equipment's surfaces from 3 plants (cheese, canned peaches and ice cream) was conducted with PFGE, according to standard operating procedures for PulseNet PFGE of *L. monocytogenes* (PulseNet, 2013).

The macrorestriction assays were performed using *Apa1* restriction enzyme. The profiles obtained were analyzed in the Gel Compar II software (Applied Maths).

3. Results

Of the 2647 total samples that came from 11 food matrices, *L. monocytogenes* was isolated in 265 samples (10%). As shown in Fig. 1, the highest prevalence was seen in red raw meat, followed by raw poultry, cured meat and sausage, and cheese prepared from pasteurized milk. *L. monocytogenes* was not detected in samples of dairy products (Heated condensed milk (N_t: 19); yogurt (N_t: 18) and pasteurized skim milk powder (N_t 43)), butter and margarine (N_t: 32), vegetables (N_t: 180) and meals (N_t: 29) such as: fruit salads (N_t: 3), lettuce salad (N_t:3), roast turkey with potatoes(N_t:5), mixed meats (N_t: 2), chicken rice and peas(N_t:5), spaghetti with tomato sauce (N_t:3), rice and beef (N_t:5), lasagna (N_t:1) and roast beef with gravy (N_t: 2).

Of the 14 positive samples from red meats, 6 were from ground beef. Of the 223 positive samples in raw poultry, 217 were from poultry purchased in retail stores (217/1103: 20%) and 6 from ground turkey.

In cured meat and sausage, *L. monocytogenes* was found from 2/37 chorizo samples studied. All samples of deli meat (N_t: 14) and hot-dogs (N_t: 11) tested negative for the pathogen.

Almost all the positive cheese samples were from two plants, named as Cheese Plant A and Cheese Plant B (94%) both Plants were visited between 2008 and 2012.

In the case of fruits and vegetables, only 1 sample tested positive and came from a fresh peach, this sample resulted positive from peeled and non-peeled fruits.

Finally, among miscellaneous food items only samples, from a pre-packaged fish patty, tested positive for *L. monocytogenes*. The miscellaneous category contained 33 samples from: evaporated condensed milk in canned (N_t: 16), frozen dough packed in bag (N_t: 6), sauce packed in plastic bag (N_t: 6), dehydrated products (N_t: 3), honey packed in plastic pot (N_t: 1) and 1 pre-packaged fish patty (N_t: 1).

The microbiological search for *L. monocytogenes* in work surfaces and equipment's performed in six processing plants associated with previous outbreaks are shown in Table 1. Of the 540 samples analyzed, *L. monocytogenes* was found in 22 (4%). In analysis of work surfaces and equipment's, 7 were found positive in the equipment's. The remaining were isolated from surfaces not necessarily in contact with the product. Surprisingly only one positive sample came from an artisanal plant while the other 6 came from industrial plants, all of which have best practices procedures in place.

In Plant A (a cheese plant), positive samples came from the table where cheese is left to mature, press and drainage systems. In Plant B (a commercial cheese) which was associated with a previous outbreak of listeriosis, *L. monocytogenes* was isolated in all final products. The pathogen was only found in samples from the floor in the processing room.

In Plant C, *L. monocytogenes* was identified on rollers during drying and after being washed with detergent and sanitizer solution.

In Plant D, samples with *L. monocytogenes* positive surfaces and equipment's were found in the area of caramel pasteurization (i.e. manjar), transport cars, tank wheel and drain grates.

In the cured meat plant, (Plant E), *L. monocytogenes* was not detected in any environmental sample, but was found in 5 out of 13 (38%) hand and fingernail samples from plant food handlers.

In Plant F, all the three positive samples (3/393) were found in line to spalling.

3.1. Evaluation of strains of L. monocytogenes through PFGE

In the cheese plant (Plant B) associated with a previous outbreak, *Listeria* strains generated just two PFGE patterns: one was shared with all strains found in the final product (4 cow's cheese flavored with oregano and 4 camembert goat's cheese) and the other generated a totally different macrorestriction pattern and came from the floor of the processing Plant. PFGE profiles were considered similar when similarity was 80% or more.

In Plant C (canned peaches), 13 strains were isolated from machines from various processing areas. Fig. 2 shows that more than one PFGE genotype was apparent in strains of *L. monocytogenes*. Dendogram analysis, considering profiles with over 80% similarity, showed that there were at least 7 different genotypes, none of which was related to the final canned peach product.

All the *L. monocytogenes* strains ($N = 3$) found in ice-cream samples, work surfaces and equipment's from Plant D had unique PFGE profile.

4. Discussion

The presence of *L. monocytogenes* is a permanent concern for the food industry, which depends on the correct application of Standard Operation Procedures (SOP) and Standard Sanitation *Operating* Procedures (SSOP), Good Manufacturing Practices (GMP) and

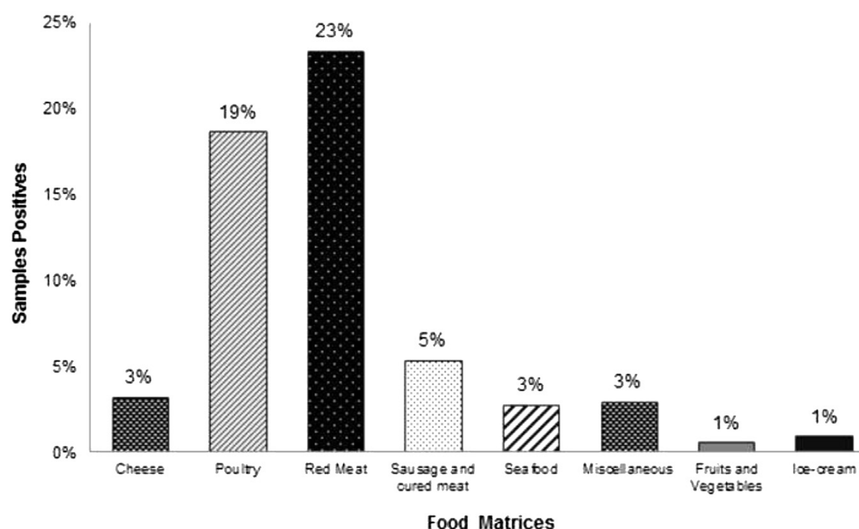

Fig. 1. Summary of isolation of *L. monocytogenes* in different food matrices between 2008 and 2012.

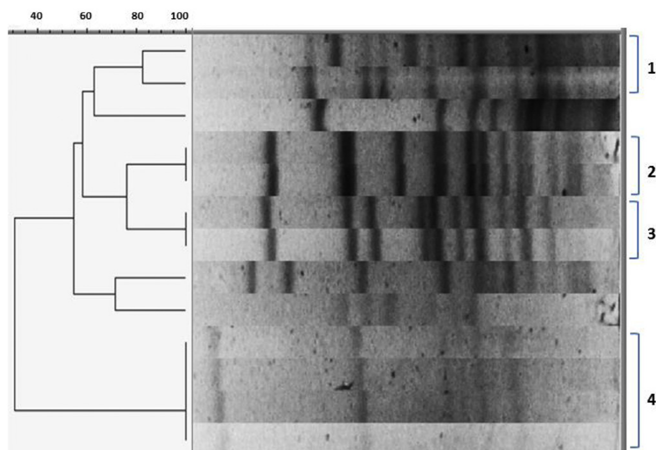

Fig. 2. PFGE dendogram of *L. monocytogenes* isolates obtained in a peach processing plant. Groups are presented for restriction profiles with more than 80% similarity over an isolated group. Group 1 corresponds to washer rollers before and after detergent cleaning, Group 2 tape and pump hose. Group 3 rollers and Group 4 to rollers before and after fungicide application and the cutting table.

HACCP. As most of the virulent bacteria *Listeria* is equipped with various mechanisms of virulence. Among them is their capacity to form biofilms, characteristic that is crucial to maintain their contamination in work surfaces, equipment's and utensils, moreover *Listeria* can reproduce at low temperature and withstand high osmolarity (Chao et al., 2006; Vázquez-Boland et al., 2001; Wałecka-Zacharska et al., 2012).

Before 2007, listeriosis was not a serious concern for health authorities in Chile because cases were sporadic and did not exceed 3 cases per million. In 2008, however, there was a large outbreak involving 165 persons (10 cases per million) associated with the intake of fresh cheese and in 2009, 73 cases were associated with consumption of meat products (Filiousis, Johansson, Frey, & Perreten, 2009). This finding have a recent and significant impact on the Chilean population and a greater need to understand which foods represent the highest risk for contamination of *L. monocytogenes*. A major strength of this study includes reporting information on the presence of *L. monocytogenes* in different food matrices in Chile. We found that the pathogen is present in the environment of food processing plants, underscoring the importance of reinforcing and applying strict systems of HACCP control to prevent contamination.

The results presented here showed that of the 6 food matrices in which *L. monocytogenes* was identified; the highest rate was found in food of animal origin (e.g. meat, sausages and cheese). This result are in line with publications from the Emerging Pathogens Institute (Batz, Hoffmann & Morris 2011) which list meat and cheese among the list of top ten foods in which *L. monocytogenes* is detected in the U.S., based on number of illnesses, hospitalizations and deaths. The high rate of *L. monocytogenes* found in this type of food is not surprising, in fact cattle and birds are frequent reservoirs for the pathogen and contamination of the product could occur during the evisceration process in meats, where *L. monocytogenes* has been detected on knifes and remain after inadequate pasteurization of cheeses, (Chao et al., 2006; EFSA, 2013; Lobacz, Kowalik, & Tarczynska, 2013) consequently both stages should be considered as a critical point in the HACCP plan. Deficiencies in the prerequisites such as Good Manufacturing Practices allows that knives and utensils in direct contact with intestinal content from birds or cattle will represents an important contamination point for the final products and the surrounding environment (Yu & Jiang, 2014).

Many studies revealed the environment as a potential contamination point during food processing. This often occurs between "raw" and "cooked" meats and contact of cooked produce with contaminated surfaces. Cross-contamination during domestic washing up has also been studied and illustrates the high potential for this to occur. Wilks, Michels, and Keevil (2006).

The origin of *L. monocytogenes* contamination in work surfaces and machines in food processing plants are usually located in areas with accumulated organic matter and high humidity. Both conditions are optimal for Listeria proliferation and the subsequent formation of biofilms (EFSA, 2013). In this study we detected the presence of *L. monocytogenes* in the washing rollers (Plant C), the tank wheel and the drainage gates (Plant D), and the press and maturity table (Plant A), a situation that could have been avoided if an appropriate cleaning with detergent followed by a proper sanitization process had been implemented (FAO/WHO 2009).

Another important factor in the contamination of products is manipulation during processing (Garayoa, Vitas, Leturia, & Jalón, 2011; Muñoz, Chaves, Rodríguez, & Realpe, 2013). In this study, *L. monocytogenes* was detected in 38% of the samples collected from hands and fingernails, indicating that food handlers may and can spread or contaminate products, as was the case of cured meats in this study.

PFGE analysis showed that more than one genotype of *L. monocytogenes* could be isolated from the work surfaces, machines and final products within the same plant. This evaluation was conducted in Plants B and C, this finding suggest that more than one point of contamination. Additionally, it is interesting that a unique profile (i.e. a single PFGE strain) seems to be present in Plant D. this result indicated that the plant had a single source of contamination that was propagated throughout the plant until contaminating the final product (ice cream).

PFGE also demonstrated that all pulsetypes obtained in the three plants were not associated with profiles detected from clones 09 and 01 of *L. monocytogenes* reported previously by the Chilean Laboratory of Disease Control (MINSAL) and involved in recent outbreaks in Chile (Foerster, Vidal, Troncoso, & Figueroa, 2012).

5. Conclusions

This study demonstrates the urgent necessity to implement prerequisites and HACCP plans to improve food safety in all high risk processing plants. The culture of Prevention is the only way to assure foods free of *L. monocytogenes* to the Chilean consumers.

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