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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.

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).