International Journal of Food Microbiology 289 (2019) 64–71

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Short communication

Listeria monocytogenes colonization in a newly established dairy processing facility

Beatriz Melero[a], Beatrix Stessl[b], Beatriz Manso[a], Martin Wagner[b], Óscar J. Esteban-Carbonero[a], Marta Hernández[a,c], Jordi Rovira[a], David Rodriguez-Lázaro[a,*]

[a] Department of Biotechnology and Food Science, University of Burgos, Burgos, Spain
[b] Institute of Milk Hygiene, Milk Technology and Food Science, Department of Veterinary Public Health and Food Science, University of Veterinary Medicine, Vienna, Austria
[c] Laboratory of Molecular Biology and Microbiology, Instituto Tecnológico Agrario de Castilla y León, Valladolid, Spain

ARTICLE INFO

Keywords:
Listeria monocytogenes
Cheese
Colonization
Persistence

ABSTRACT

The presence and colonization of *Listeria monocytogenes* were investigated in a newly established dairy processing plant during a one-year period. A total of 250 non-food contact surfaces, 163 food contact surfaces, 46 personnel and 77 food samples were analyzed in two different buildings according to the cheese production chain. Initial steps, including salting, are performed in building I (old facility), while the final steps, including ripening, cutting and packaging, are performed in building II (new facility). Overall, 218 samples were collected from building I and 318 from building II. *L. monocytogenes* isolates were subtyped by PFGE and MLST, and a questionnaire about quality measures was completed. The overall prevalence of *L. monocytogenes* was 8.40%, and while the presence of the pathogen was observed just during the first sampling in building I, *L. monocytogenes* was found in building II at the third sampling event. The salting area in building I had the highest proportion of positive samples with the highest diversity of PFGE types. Moreover, *L. monocytogenes* PFGE type 3 (sequence type -ST- 204) was first detected in building II in the third visit, and spread through this building until the end of the study. The answers to the questionnaire implied that lack of hygienic barriers in specific parts of the facilities and uncontrolled personnel flow were the critical factors for the spread of *L. monocytogenes* within and between buildings. Knowledge of the patterns of *L. monocytogenes* colonization can help a more rational design of new cheesemaking facilities, and improve the food safety within current facilities.

1. Introduction

Listeria monocytogenes is a ubiquitous bacterium that can be isolated from a wide variety of environmental sources, including food-processing environments and a large variety of foods where it can grow over a pH range of 4.39–9.40, even at refrigeration temperatures (Gandhi and Chikindas, 2007; Sauders and Wiedmann, 2007; Swaminathan and Gerner-Smidt, 2007). In 2016, 2536 human cases were reported in 28 EU member states, causing by far the highest number of food-borne diseases-related deaths (EFSA, 2017). Food safety regulations in many countries such as the USA, have tended to adopt a zero tolerance policy for *L. monocytogenes* in ready-to-eat (RTE) food products, as human listeriosis outbreaks have been most often associated with RTE products that are consumed without prior cooking (Painter and Slutsker, 2007; Swaminathan and Gerner-Smidt, 2007). Cheese and other dairy products are within this type of food category. Unlike many other bacterial foodborne pathogens, *L. monocytogenes* can grow in milk at refrigeration temperatures (Kozak et al., 2018; Thamnopoulos et al., 2018) and reach potentially infectious levels in high-moisture and surface-ripened cheeses (Bernini et al., 2013; Cogan, 2011). The survival and growth of *L. monocytogenes* in dairy environments depends on the manufacturing, ripening and storage conditions (Almeida et al., 2013; Pintado et al., 2005). Similarly, the strain-to-strain variability of survival in different storage conditions is associated to the different *L. monocytogenes* genetic lineages (De Jesus and Whiting, 2003; Mataragas et al., 2008).

Although *L. monocytogenes* can decrease in different types of cheeses during ripening and storage (Valero et al., 2014; Wemmenhove et al., 2013), the risk of cross-contamination during processing is still high due to the possible presence of this organism in the dairy environment

* Corresponding author at: Microbiology Division, Department of Biotechnology and Food Science, University of Burgos, Plaza Misael Bañuelos, 09001 Burgos, Spain.
E-mail address: drlazaro@ubu.es (D. Rodriguez-Lázaro).

https://doi.org/10.1016/j.ijfoodmicro.2018.09.003
Received 30 April 2018; Received in revised form 30 August 2018; Accepted 3 September 2018
Available online 05 September 2018
0168-1605/

(Muhterem-Uyar et al., 2015; Rückerl et al., 2014; Spanu et al., 2015). Failures of hygiene practices or the incorrect design of equipment or facilities may facilitate *L. monocytogenes* presence and persistence in cheese making facilities (Almeida et al., 2013; Carpentier and Cerf, 2011; Fox et al., 2011; Ibba et al., 2013). Upon colonization of facilities, *L. monocytogenes* can spread easily via contaminated contact materials, inappropriate personnel movements and food workflows, which can constitute an intermediate step in transmission from their original habitat (in biofilms, water and organic soil residues) to surfaces in contact with foods (Muhterem-Uyar et al., 2015; Stessl et al., 2014). Thus, tracing the presence and persistence of *L. monocytogenes* in food commodities is a major issue in food safety.

The aim of this study was to describe the process of colonization by *L. monocytogenes* of a newly opened (6 months), *Listeria*-free dairy processing plant for first samplings by the investigation, during a single year, of the presence and persistence of *L. monocytogenes* and the characterization of its possible routes of contamination.

2. Materials and methods

2.1. Sampling strategy

The presence and persistence of *L. monocytogenes* was investigated in a recently inaugurated dairy processing facility in Castilla y Leon, Spain during a one-year period, in ten different visits from November 2012 to November 2013 every one month and a half. Cheese is produced in two different buildings, 13 km apart (Fig. 1). Building I, which started production in 1984, comprises the first production steps such as 1) milk reception, procession and curdling; 2) salting; and 3) palletizing (crates with unripened cheeses are stacked on a pallet to be transported to the ripening station). Building II, which started production in May 2012, includes the final production steps such as 4) ripening; 5) slicing and modified atmosphere packaging; and 6) cheese grating (Fig. 1). Building II also received cheeses from two other company's plants, located in other regions in Spain.

The selection and number of samples and sites followed a previous described sampling strategy, with a scope of collecting 50 samples per sampling event (Muhterem-Uyar et al., 2015). The sample sites were the same in all the sampling events along the study, taken during the working shift and before cleaning and disinfection. Environmental samples (food contact surfaces –FCS- and non-food contact surfaces -NFCS-), pasteurized cheese, and product-associated samples like brine were tested during the processing. A total of 536 samples were taken in both buildings (218 in the building I and 318 in the building II); comprising 250 NFCS, 163 FCS, 46 personnel and 77 food samples. An average of 53.6 samples per sampling day was collected. Table 1 summarizes all the samplings that were carried out according to the building, production step, sample type and visit. Both the FCS and the NFCS were investigated by swabbing at least 900 cm^2 areas with sterile sponges moistened with 10 mL of buffered peptone water (3 M, St. Paul, MN, USA). All samples were maintained at 4 °C during transportation to the laboratory and were analyzed for the presence of *L. monocytogenes* within 24 h.

2.2. Detection of Listeria monocytogenes

The presence of *L. monocytogenes* in environmental swabs, liquids and solid food samples was investigated as described previously (Muhterem-Uyar et al., 2015). One confirmed *L. monocytogenes* colony by real-time PCR (Rodríguez-Lázaro et al., 2004), was used for further genetic characterization.

2.3. Questionnaire

A questionnaire about personnel behaviour, plant infrastructure and hygienic and cleaning measures was completed by the Quality Manager from each building, to provide a better knowledge of contamination routes (Table 2).

2.4. Genetic characterization

L. monocytogenes PCR-serogrouping was performed using a multiplex PCR as previously described (Doumith et al., 2004). All isolates were genetically characterized by pulsed-field gel electrophoresis (PFGE) using the restriction enzymes *Asc*I and *Apa*I following the standardized PulseNet protocols as previously described (Rodríguez-Lázaro et al., 2015). In the framework of this study, a *L. monocytogenes* strain was defined as persistent when an identical PFGE type was detected at least 6 months apart from the first time. *L. monocytogenes* isolates were further characterized by multilocus sequence typing (MLST) as previously described (Rodríguez-Lázaro et al., 2015).

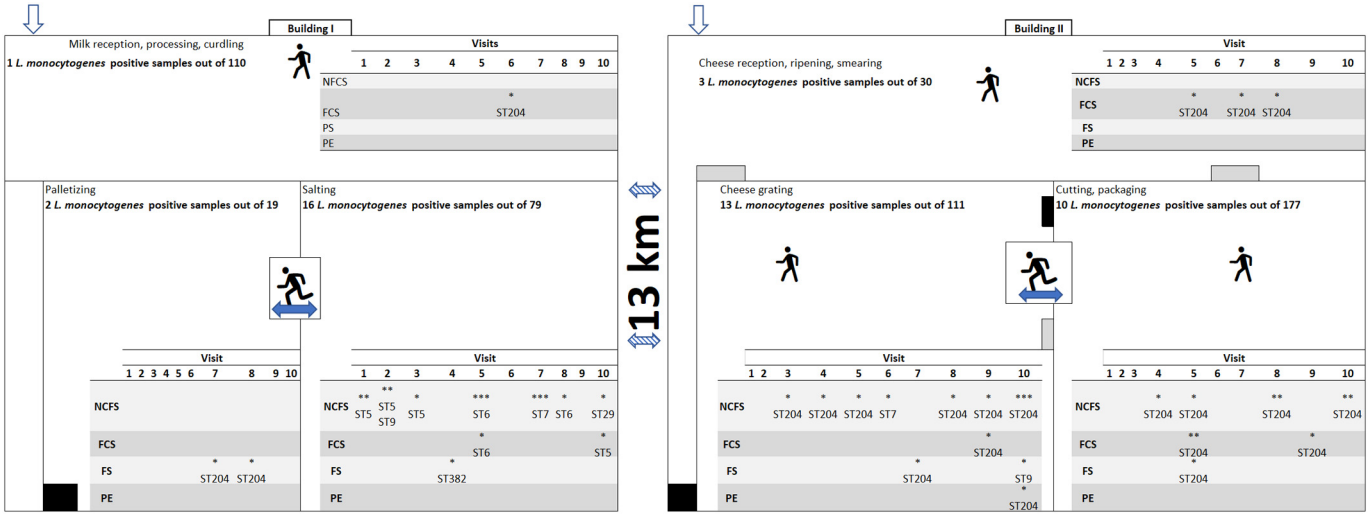

Fig. 1. Schematic representation of the two buildings (located 13 km apart) and the production process/flow of the dairy plant analyzed in this study. Different types of entrances are marked by gray (hygienic) or black (non-hygienic) rectangles. Personnel for which movement is restricted are indicated in black, while personnel moving within different areas are indicated inside a square and with an arrow indicated the direction. An empty arrow indicates the entrance of product in each building. NFCS (Non-food Contact Surfaces); FCS (Food Contact Surfaces); FS (Food Samples); PE (Personnel). * number of positive samples for each visit and each sample type. ST, multi-locus sequence type.