BRAZILIAN JOURNAL OF MICROBIOLOGY XXX (2017) XXX–XXX

BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Food Microbiology

Prevalence and serotype distribution of *Listeria monocytogenes* isolated from foods in Montevideo-Uruguay

Valeria Braga[a,b], *Sylvia Vázquez*[a], *Victoria Vico*[b], *Valeria Pastorino*[a], *María Inés Mota*[b], *Marcela Legnani*[a], *Felipe Schelotto*[b], *Gustavo Lancibidad*[a], *Gustavo Varela*[b,*]

[a] *Laboratorio de Bromatología – Intendencia de Montevideo, Isla de Flores 1323, PC 11800, Uruguay*
[b] *Departamento de Bacteriología y Virología – Instituto de Higiene, Facultad de Medicina – UdelaR, Alfredo Navarro 3051, PC 11600 Montevideo, Uruguay*

ARTICLE INFO

Article history:
Received 12 September 2016
Accepted 12 January 2017
Available online xxx

Associate Editor: Luis Augusto Nero

Keywords:
Food-borne diseases
Listeriosis
Serotyping
PFGE

ABSTRACT

The aim of this work was to study the prevalence of *Listeria monocytogenes* in foods obtained in retail shops and food industries located in Montevideo-Uruguay, and to identify the serogroups of the obtained isolates. Three-thousand one-hundred and seventy-five food samples (frozen, deli meats, ready-to-eat and cheese) were analyzed. The obtained isolates were serogrouped by multiplex PCR and serotyped by conventional procedure. Genetic comparisons were performed using pulsed-field gel electrophoresis on a sub-set of isolates belonging to the same serotype successively recovered from the same establishment. *L. monocytogenes* was isolated from 11.2% of samples. The highest prevalence was observed in frozen foods (38%), followed by cheese (10%). 1/2b and 4b were the most frequently identified serotypes. In six of 236 analyzed establishments we successively recovered *L. monocytogenes* isolates belonging to the same serotype. Most of them corresponded to serotype 1/2b. Pulsed-field gel electrophoresis profiles suggest that at least 33% of *L. monocytogenes* 1/2b isolates are genetically related and that may remain viable for prolonged periods. The observed prevalence of *L. monocytogenes* was lower than reported in neighboring countries. Our findings highlight the role that frozen foods may play in the spread of this pathogen, and the relevance of serotypes 1/2b and 4b.

* *Corresponding author at*: A. Navarro 3051, PC 11600 Montevideo, Uruguay.
E-mail: gvarela@higiene.edu.uy (G. Varela).

http://dx.doi.org/10.1016/j.bjm.2017.01.010

Please cite this article in press as: Braga V, et al. Prevalence and serotype distribution of *Listeria monocytogenes* isolated from foods in Montevideo-Uruguay. *Braz J Microbiol.* (2017), http://dx.doi.org/10.1016/j.bjm.2017.01.010

Introduction

Listeria monocytogenes is a bacterial pathogen that causes a potentially severe disease both in people and animals, called listeriosis. Worldwide, it has been estimated that almost 90% of human cases occur after consumption of contaminated food. The most frequently implicated foodstuffs both in outbreaks and sporadic cases are soft cheese, frankfurters, unpasteurized milk, deli meats, smoked fish, dairy products, salads and refrigerated ready-to-eat products (RTE).[1–5]

Listeriosis may occur as a mild febrile gastroenteritis or as a more severe invasive disease such as meningoencephalitis or sepsis. *L. monocytogenes* is capable of producing serious illness in pregnant women, newborns, elderly people and immunocompromised individuals (e.g. transplant recipients, patients receiving antineoplastic drugs or immunosuppressive therapy). In this high-risk group, listeriosis has a high lethality rate of 20–30%.[4–7]

This bacterium is widely disseminated in different food production environments, including manufacturing and packing plants, distribution and storage facilities. It develops biofilms on equipment, walk-in freezers, drains, etc.[8–10] This feature, added to its capacity for growth at cooling temperatures and survival through long freezing–thawing periods[11] are responsible for the increasing risks of food contamination with this bacterium.

Since 1980s, *L. monocytogenes* has emerged as a major public health burden. Nevertheless, its importance as a food-borne pathogen is not always recognized, due to a very long incubation period between consumption of the contaminated food and onset of illness as well as sporadic exposure. Nowadays *L. monocytogenes* represents a serious challenge to food safety and it is one of the microorganisms of significant concern the food industry.[12–14]

While several phenotypic and genotypic methods have been described and applied to the study of *L. monocytogenes* isolates, the most frequently used are serotyping and DNA macro-restriction digest followed by pulsed-field gel electrophoresis (PFGE).[15–23] PFGE remains as the gold standard method applied to the study of listeriosis outbreaks.[19–22,24]. Serotyping is an additional tool to characterize *L. monocytogenes* isolates.[23] To date, 13 serotypes have been defined for *L. monocytogenes*. More than 90% of the recovered strains from food, animal and human samples typically belong to serotypes 1/2a, 1/2b, 1/2c and 4b. The multiplex PCR assay previously described by Doumith et al.,[23] appears as practical screening procedure to identify these serotypes.

PFGE remain as the gold standard method applied to the study of listeriosis outbreaks.[19–22,24]

The purposes of this work were to study the prevalence of *L. monocytogenes* contamination in various food samples obtained from retail store and food industries located in Montevideo city, Uruguay and to identify the main serotypes of the obtained isolates.

Materials and methods

Processing of food samples

Between October 2011 and August 2013, 3175 food samples were analyzed (220 were frozen products, both from animal and vegetable source; 2180 ready-to-eat foods, from animal and vegetable origin; 580 deli meats; and 195 cheese) at the Bromatological Laboratory (Intendencia de Montevideo). Samples were taken from 236 establishments (retail shops and food industries) located in Montevideo city, Uruguay as part of a food safety surveillance program.

For microbiological studies, pools of five products from the same establishment and food category were prepared (44 of frozen products, 436 of ready-to-eat foods, 116 of deli meats, and 39 of cheese, respectively) and were analyzed according to ISO 11290-1-1996 and ISO 11290-1:1996/Amd 1:2004 guide[25,26] using a real-time PCR DuPont Qualicon BAX® System (DuPont Qualicon, Wilmington, DE, USA) following the manufacturer's instructions.

L. monocytogenes isolation and identification

Positive samples according to real-time PCR results were streaked onto two selective media plates: Oxford medium base with Modified Oxford Antimicrobic Supplement (BD – Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and BBL™ CHROMagar™*Listeria* (BD), both incubated for 24–48 h at 35–37 °C. Suspect colonies (small, convex and surrounded by a black halo; blue colonies less than 3 mm in diameter and surrounded by a white halo, respectively) were identified using API *Listeria* kit (bioMérieux, Marcy l'Etoile, France) and additional standard biochemical assays that included: β-Hemolysis halo production on Trypticase Soy Agar (BD) supplemented with sheep blood (5%) plates, catalase reaction, bile esculin; and Christie-Atkins-Munch-Petersen (CAMP) tests with control strains of *Staphylococcus aureus* and *Rhodococcus equi*.[27]

When food samples from retail shop or factory displayed positive results to *L. monocytogenes*, it was visited again and samples of the same food types were taken and analyzed according to the above described procedure.

Confirmed *L. monocytogenes* isolates were conserved in 10% reconstituted skim milk at −20 °C and also in 20% glycerol at −80 °C for further assays.

Serotyping

Multiplex PCR was used to determine *L. monocytogenes* serogroup as described previosly.[23] Briefly, DNA was extracted from colonies grown overnight in Trypticase Soy Agar plates (BD) supplemented with sheep blood (5%). Colonies (4–5) were suspended in 50 μL of lysis buffer (SDS 0.25%, NaOH 0.05 N) and boiled for 15 min. Then, 100 μL of ultra-purified water was

Please cite this article in press as: Braga V, et al. Prevalence and serotype distribution of *Listeria monocytogenes* isolated from foods in Montevideo-Uruguay. *Braz J Microbiol.* (2017), http://dx.doi.org/10.1016/j.bjm.2017.01.010