# *Listeria monocytogenes* IN HOT DOG SAUSAGES OBTAINED FROM GROCERIES STORES IN THE CITY OF SÃO PAULO – A COMPARATIVE AND RETROSPECTIVE ANALYSIS OF HUMAN LISTERIOSIS ISOLATES

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## ABSTRACT

Three hundred and ninety four hot dog samples obtained from retail markets (groceries stores) in the city of São Paulo/Brazil were investigated for the presence of *Listeria monocytogenes* and for the respective serovars of the isolated strains. A total of 56 strains of *L. monocytogenes* were found. Serovars 1/2a and 1/2c were each identified in 41.2% of the samples (14/34) and the serovar 4b was found in 17.6% (6/34). It was concluded that *Listeria monocytogenes* occur in hot dogs sold at these markets in the city of São Paulo; it was predominating in the hot dogs samples serovars: 1/2a (41.2%) and serovar 1/2c (41.2%), followed by serovar 4b (17.6%); the serovars isolated from hot dog samples were the same ones associated to outbreaks and sporadic cases including serovar 4b, even though it showed a lower percentage against the others serovars detected; it must be considered as routine practices the monitoring of the thermal treatment during the cooking process of hot dogs, the evaluation of the occurrence level of *L.monocytogenes* on bench surfaces and equipment used during post-thermal treatments, it must be realized the re-pasteurization after packaging; it is very important to implement the Good Manufacturing Practices – GMP and the Hazard Analysis and Critical Control Point – HACCP in the food industries, restaurants and any food production areas.

**Key words** : hot dog sausages; *Listeria monocytogenes*; good manufacturing practices; hazard analysys and critical control point.

# *Listeria monocytogenes* EM AMOSTRAS DE SALSICHA TIPO HOT DOG COMERCIALIZADAS EM SUPERMERCADOS DA CIDADE DE SÃO PAULO – ANÁLISE COMPARATIVA E RETROSPECTIVA COM OS SOROVARES ASSOCIADOS A LISTERIOSE HUMANA

## RESUMO

Pesquisou-se *Listeria monocytogenes* em 394 amostras de salsichas tipo hot dog, comercializadas em supermercados da cidade de São Paulo, com posterior identificação dos sorovares. Isolou-se 56 estirpes de *L.monocytogenes* que ocorreram em 55,4% das amostras. Os sorovares 1/2a e 1/2c ocorreram igualmente em 41,2% (14/34) e o sorovar 4b ocorreu em 17,6% (6/34). Esses sorovares (1/2a, 1/2c e 4b) já foram relacionados com surtos ou casos esporádicos de listeriose humana, não apenas no Brasil, mas em outros países. Deve-se,

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portanto, monitorar o tempo e a temperatura de cozimento da salsicha e manter sistemas de avaliação da ocorrência de *L.monocytogenes* em equipamentos e utensílios que estão sempre em contato com o produto pós-tratamento térmico. Deve-se, igualmente, proceder a repasteurização após a embalagem, fazendo-se indispensável a implementação de boas práticas de fabricação de alimentos – BPF e do sistema Análise de Perigos e Pontos Críticos de Controle – APPCC nas indústrias de alimentos, restaurantes e qualquer outro setor de produção de alimentos.

**Palavras-chave**: salsichas tipo hot dog, *Listeria monocytogenes*, boas práticas de manipulação; análise de perigos e pontos críticos de controle.

## *Listeria monocytogenes* EN MUESTRAS DE SALCHICHA TIPO HOT DOG COMERCIALIZADAS EN SUPERMERCADOS DA LA CIUDAD DE SÃO PAULO – ANÁLISIS COMPARATIVA Y RETROSPECTIVA CON LOS SEROVARES ASOCIADOS A LISTERIOSIS HUMANA

### RESUMEN

Se investigó *Listeria monocytogenes* en 394 muestras de salchichas tipo hot dog, comercializadas en supermercados de la ciudad de São Paulo, y posterior identificación de los serovares. Se aisló 56 estirpes de *L.monocytogenes* que ocurrió en el 55,4% de las muestras. Los serovares 1/2a y 1/2c ocurrieron igualmente en el 41,2% (14/34) y el serovar 4b ocurrió en el 17,6% (6/34). Esos serovares (1/2a, 1/2c y 4b) ya fueron relacionados con brotes o casos esporádicos de listeriosis humana, en Brasil y otros países; el monitoraje del tiempo y temperatura de cocimiento de la salchicha debe ser rutinario, se debe mantener sistemas de evaluación de la ocurrencia de *L.monocytogenes* en equipos de cocina y utensilios que mantienen contacto con el producto pos-tratamiento térmico; se debe proceder a la re-pasteurización después del embalaje y se hace indispensable la implementación de buenas prácticas de fabricación de alimentos – BPF y del sistema Análisis de Peligros y Puntos Críticos de Control – APPCC en industrias de alimentos, restaurantes y cualquier otro sector de producción de alimentos.

**Palabras-clave:** salchichas tipo hot dog, *Listeria monocytogenes*,

## INTRODUCTION

*Listeria monocytogenes* is a ubiquitous bacterium with the ability to proliferate at refrigerated temperatures and under adverse environmental conditions with little nutrients. These properties hamper the elimination of this pathogen in food processing industries since this microorganism can survive for long periods in drainers, condensation on walls and ceilings, water droplets on the floor, cleaning utensils, and equipment used during the production phase (BECKWITH, 1990).

The incidence of listeriosis has been increasing in several countries and epidemiological studies have shown that both, sporadic and outbreak cases, are mainly due to the transmission of contaminated processed foods such as milk and meat based products, vegetables, fish and ready-to-eat foods or cold pre-prepared foods (SNELLING et al., 1991).

The American Center for Diseases Control and Prevention (CDC) estimates that there are 2500 cases of listeriosis annually in the USA. This number is apparently low when

compared to other diseases transmitted by processed foods; however, the mortality rate caused by listeriosis is estimated to be greater than 20% (500 death/year). Therefore, studies of listeriosis are of great importance to better understand its transmission and to develop appropriate prevention and disease control methods (FOOD SAFETY EXPRESS, 2000).

The severity of the listeriosis and its socio-economical consequences vary for different ethnic groups who live under diverse conditions. *L.monocytogenes* can cause various degrees of illness and its dissemination is due to the contamination of raw or processed foods. It is difficult to control the transmission of this pathogen, and its incidence should be prevented at all stages of the food production chain, i.e., from the production of the raw materials (vegetables and meats) to the consumer by management and by proper handling of food items.

Children, elderly, pregnant women, sick, and convalescencing people are at a greater risk of listeriosis since their immune systems are either compromised or not fully functional. Foods developed for the growing market of processed, cooked or ready-to-eat food items, which are usually stored at refrigerated temperatures, are increasingly associated with the psychrotrophic nature of *L.monocytogenes* and require improved control measures from both, the food industries and from public health entities (LOGUERCIO et al., 2001).

The consumption of hot dogs, mainly by children and adolescents, is higher in big cities due to their convenience and low price. However, hot dogs are suitable carrier of *L.monocytogenes*, i.e., they are ready-to-eat meat-derived products, which are maintained under refrigeration and require handled after industrial thermal processing.

The objectives of this study were to evaluate the presence of *L.monocytogenes* in samples of hot dog from the city of São Paulo, SP, Brazil, followed by the identification of serovars and their comparison with those detected in sporadic cases and outbreaks of human listeriosis reported in the literature.

## MATERIAL AND METHODS

A total of 394 hot dog samples from six different brands obtained from supermarkets located in the city of São Paulo, SP, Brazil, were analyzed from June 2001 to October 2002, utilizing the *Listeria* Immunoassay Visual Tecra® (LISVIA) kit. The use of this kit was approved by the *Association of Official Analytical Chemists* 995.22 and its use in Brazil was authorized by the Department of Agriculture, Cattle and Resource (MA – 21000.001973/98-69). This assay is an immunoenzymatic test – ELISA - *Enzyme-linked immunosorbent assay* that uses the sandwich configuration with visual reading.

Positive samples for *Listeria* spp were analyzed biochemically to confirm the presence of *L.monocytogenes*, while negative samples were those that showed no *Listeria* spp in 25 g/sample. Samples that showed a positive LISVIA kit reaction were streaked on Petri plates containing modified Lithium Chloride-Phenylethanol-Moxalactam agar (LPM) supplemented with 0.1% of esculin and 10% citrate ferric ammoniacal and Palcam agar. Inoculated plates were incubated at 30ºC (± 1ºC) for 48 h without light.

Isolated colonies were transferred to slant tubes containing Soybean Trypticase Agar (STA) amended with 0.6% yeast extract (YE) and incubated as mentioned above. Subsequently, the presence of *L.monocytogenes* in the cultures was confirmed through the following biochemical tests: 1) *CAMP* test (Christie-Atkins-Munch-Petersen): the presence of *L.monocytogenes* causes a positive CAMP reaction; 2) Hemolysis test: *L.monocytogenes* is β-hemolytic; 3) Motility test: positive samples show a migration zone around the streaked area with an umbrella shape as the pathogen grows; 4) Triplice Sugar Iron Agar reaction test (TSI): *L. monocytogenes* produces acid by fermenting the glucose present in the culture medium,

thus, altering the original red color to yellow without producing $H_2S$; 5) Sugars fermentation test: *L.monocytogenes* ferments rhamnose, maltose, a-metyl D-manoside, but not manitol and xylose, and therefore, it does not alter the culture medium color, and 6) Catalase assay: *L.monocytogenes* produces catalase.

The determination of the antiserum of the serogroups was conducted according to the procedure of serum agglutination on slides (SEELIGER & HOHNE, 1979), by using somatic and flagellar polyclonal and monoclonal antisera produced at the Bacterial Zoonose Laboratory from the 'Fundação Instituto Oswaldo Cruz', Rio de Janeiro, RJ, Brazil.

## RESULTS AND DISCUSSION

**Table 1 –** Number and percentage of positive samples for *Listeria* spp and *L.monocytogenes* per brand of hot dog sausage analyzed, and the ratio of *L.monocytogenes* to *Listeria* spp – São Paulo, 2006.

| brand | n° samples | *Listeria* spp / brand n° / % | *L. monocytognes* / brand n° / % | *L. monocytogenes* / *Listeria spp* |
|---|---|---|---|---|
| A | 20 | 6 (30%) | 1 (5%) | 1 / 6 (16%) |
| B | 35 | 11 (31%) | 7 (20%) | 7 / 11 (63%) |
| C | 15 | 1 (6%) | 0 (zero) | 0 / 1 (zero) |
| D | 128 | 0 (zero) | 0 (zero) | 0 / 0 (zero) |
| E | 80 | 29 (36%) | 16 (20%) | 16 / 29 (55%) |
| F | 116 | 54 (46%) | 32 (20%) | 32 / 54 (59%) |
| **Total** | **394** | **101 (25%)** | **56 (14%)** | **56 (55%)** |

Of the 394 hot dog samples analyzed, 101 (25.6%) were contaminated with *Listeria* spp. This contamination was on the surface and was caused during the industrial processing since the samples were obtained from their original unopened packages, and only the surface parts were analyzed. Moreover, the high contamination rate observed was probably due to the ubiquitous nature of this pathogen, which has different species, and to the product that is made from non-sterile ingredients and raw materials.

These results corroborate with data from the literature for the presence of *Listeria* spp in meat-derived cooked product or in those that underwent thermal treatment, such as pasteurization, during the industrial processing, followed by manual manipulation before or after final packaging. Chunhua et al. (1994) showed that 10% of 93 hot dog samples examined were contaminated with *Listeria* spp, while Bersot et al. (1998) found that 36.7% of 30 sliced bolony samples had *Listeria* spp.

Little was found in the literature about the isolation of *Listeria* spp. It appears that most studies were concerned with *L.monocytogenes* and the determination of its concentration per weight unit or volume of product. Our results showed that of the 101 bacteria strains isolated, 56 (55.4%) were confirmed to be *L.monocytogenes*. Several authors have isolated *Lmonocytogenes* from different environments, equipment and working tools (HOFER, 1975; SCHLECH et al., 1983; HOFER et al., 1984; BECKWITH, 1990; ROCOURT et al., 1997; RODRIGUES et al., 1999; TRABULSI et al., 1999; HOFER et al., 2000) as well as from animal and human feces (HOFER, 1974; 1983; RALOVICH, 1984; TRABULSI et al., 1999).

It is worth mentioning that *L.monocytogenes* is widely distributed in nature and has been isolated from processed raw or *in natura* meat-derived products (CASERIO et al., 1989;

LAGE, 1993; SERAFINI, 1993; SILVA, 1996) at rates that varies from 1.7% to 11.9% depending on the product.

Fast identification methods employed to detect microorganisms of public health importance is of great value for the food industry. In general, negative results for *L.monocytogenes* would allow for a faster and safer release of food products for the consumers, while positive results for this pathogen would prevent the release of these contaminated products and could help accurately determine the sources of the contamination for that particular food lot. The fast methods allow positive and negative results to be acquired within 50 h, while conventional methods can take from 96 to 120 h.

It's possible the occurrence rate of *L.monocytogenes* found in cooked products varies due to the following: 1) the intensity of contamination that the product was exposed to during manipulation after thermal processing; 2) the quality of the hygienic conditions of the working equipment, machines, benches and tools that were in contact with the product after thermal treatment, and 3) the combination of intrinsic and extrinsic factors of the products that can contribute to the survival and/or growth of this pathogenic agent in the final. Moreover, sample sizes, the methodologies used for the isolation of *Listeria* spp, the number of colonies that are selected and analyzed for phenotypic tests are parameters of disagreement between authors to estimate the occurrence frequency rate of *L.monocytogenes* (FABER et al., 1991).

More than one *Listeria* species can be isolated and identified from the same sample since the ecological niche of several species probably overlap (LAGE, 1993; LAWRENCE et al., 1994 apud ARAÚJO et al., 2002). Faber et al. (1989) reported the identification of 19 colonies of *L.innocua* and one of *L.monocytogenes* out of 20 colony-forming units obtained from a beef analysis.

Our study showed the predominance of *L.monocytogenes* serovars 1/2a, at 41.2% (14/34), and 1/2c also at 41.2% (14/34) over the serovar 4b, which had an occurrence frequency rate of 17.6% (6/34). These data corroborate with those reported in the literature, i.e., serovars 1/2a, 1/2b, 1/2c are the most common isolates found in meat-derived products (JAY, 1996). Serovar 4b has also been a common isolate detected in these products (SERAFINI, 1993; GILOT et al., 1996; SILVA, 1996; ROCOUT, 1997; DURANGO et al., 2001; FORSYTHE, 2002).

On the other hand, our results did not agree with those reported by Nicolas et al. (1989 apud Hofer); Faber & Peterkin (1991); McLauchlin (1997) and Ribeiro et al.(2000). These authors documented the predominance of serovar 1/2 and the presence of 1/2c in different food products. In the USA and Canada, serovars 1/2b and 1/2c are the most common isolates found in food products (FABER et al., 1991; HOFER et al., 2000).

*L.monocytogenes* serovar 4b is the most common isolate involved in human and animal diseases (ESPER, 1978; SCHLECH, 1983; JAMES, 1985; BANNISTER, 1987; GILOT et al., 1996; ROCOUT, 1996; McLAUCHLIN, 1997 HOFER et al., 1998; HOFER et al., 1999; FDA, 2003) as well as in outbreaks and sporadic cases (SCHLECH et al., 1983; JAMES, 1985; BANNISTER, 1987; GILOT et al., 1996; FDA, 2003), even though serovars 1/2a, 1/2b and 1/2c have been detected in cases of human listeriosis (BARNES, 1989; McLAUCHLIN, 1997).

In Brazil, studies have shown that serovar 4b is the most prevalent followed sequentially by 1/2b and 1/2a, and 1/2c in meat-derived products (SERAFINI, 1993; SILVA, 1996; HOFER et al., 2000; SILVA et al., 2001). *L.monocytogenes* serovars 4b and 1/2a were reported in sporadic cases to be the main causal agents of human listeriosis in immune deficient people (ESPER et al., 1978; HOFER et al., 1998 and 1999). Hofer (1975, 1983 and 1984) reported the presence of *L. monocytogenes* in samples obtained from several

environment places, including sewage and different types of materials. An environmental survey conducted by Hofer et al. (2000) showed that *L. monocytogenes* 1/2b (30.5%) was the predominant serovar followed by 4b (25.5%). They concluded that the environment could act as a reservoir and source of *L.monocytogenes*, thus contaminating working surfaces, equipment, raw materials, reagents, and work tools. Moreover, Beckwith (1990) found *L.monocytogenes* on floors, walls, ceilings, cleaning materials such as sponges and mops, and in equipment used to process hot dogs.

According to Faber et al. (1996), *L.monocytogenes*-free food products are difficult to obtain due to its capacity to produce bio-films, however, its control and the reduction of the contamination rate should be a constant practice. Although, pasteurization can inactivate this pathogen, ready-to-eat products such as hot dogs can be contaminated with *L.monocytogenes* at the packing plant or due to incorrect handling of the product at any processing step following thermal treatment up to consumption. Once the hot dogs are contaminated, this agent will survive and multiply at refrigeration temperatures. Therefore, rigorous procedures to monitor the presence of this pathogen in ready-to-eat products that are exposed to the environment or are manipulated post-thermal treatment must be implemented by public health entities.

According to Piyeasena et al. (1999), *L. innocua* could be used as a probe to estimate the thermal tolerance degree of *L.monocytogenes* in food products since the former agent has a higher tolerance rate to heat at the same assay conditions (FOEGEDING et al., 1991; FAIRCHILD et al., 1993; KAMAT et al., 1996; PIYEASENA et al., 1998).

Due to the difficulties encountered to control the contamination by *L.monocytogenes*, public health entities should enforce the use of procedures to monitor the presence of *L.monocytogenes* in food products, workstations and equipment used during food processing steps as well as for sanitation measures for food industries, restaurants and any food production areas. These procedures and measures should be implemented within programs to adequately prepare industrial food products associated with standard food processing operations. Moreover, these should be undertaken as a progressive program with the implementation of plans to analyze critical steps to control the risk of contamination during food processing.

The monitoring of the thermal treatment during the cooking process of hot dogs, the evaluation of the occurrence level of *L.monocytogenes* on bench surfaces and equipment used during post-thermal treatments, and pasteurization after packaging of hot dogs should be conducted and considered as routine practices.

## CONCLUSIONS

*Listeria monocytogenes* occure in the hot dogs market (groceries stores) in the city of São Paulo;

It was predominating in the hot dogs samples serovars: 1a (41.2%) and serovar 1c (41.2%), followed by serovar 4b (17.6%);

The serovars isolated from hot dog samples were the same ones associated to outbreaks and sporadic cases including serovar 4b, which is associated to 70% of the cases of human listeriosis, even though it showed a lower percentage against the others serovars detected;

It must be considered as routine practices the monitoring of the thermal treatment during the cooking process of hot dogs, the evaluation of the occurrence level of

*L.monocytogenes* on bench surfaces and equipment used during post-thermal treatments, and the product pasteurization after packaging;

It's very important to implement the Good Manufacturing Practices – GMP and the Hazard Analysis and Critical Control Point – HACCP in the food industries, restaurants and any food production areas.

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