THE INCIDENCE AND LEVEL OF *LISTERIA* SPP. AND *LISTERIA MONOCYTOGENES* CONTAMINATION IN PROCESSED POULTRY AT A POULTRY PROCESSING PLANT

CLÁUDIA A.C. LOURA¹, ROGERIA C.C. ALMEIDA¹ AND PAULO F. ALMEIDA²,³

¹Departamento de Ciência dos Alimentos da Escola de Nutrição
²Departamento de Ciências da Biointeração do Instituto de Ciências da Saúde
UFBA, Av. Reitor Miguel Calmon, s/n.
Canela, CEP 40110-160
Salvador, BA, Brazil

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ABSTRACT

To estimate the incidence and levels of *Listeria* spp. in an industrial poultry processing plant, samples of chicken breast meat, livers, surfaces of saws and tables, hands and gloves were analyzed. Forty percent of the breast samples presented *Listeria*:* L. monocytogenes*, *L. innocua* and *L. grayi*. Liver samples were contaminated by *L. innocua* and *L. grayi*. High levels of *Listeria monocytogenes* were found on saws (<30–2400 MPN/equipment) and tables (<30–11,000 MPN/equipment). Hands were contaminated by *L. monocytogenes*, *L. innocua* and *L. grayi* and gloves with *L. innocua* and *L. grayi*. The levels of *Listeria* on hands and gloves were low (<110 MPN/hand). *L. monocytogenes* serotypes were 1/2a, 1/2b, 1/2c and 4b. Overall, the study demonstrated the high prevalence of *Listeria* spp. and specifically *L. monocytogenes* in chicken breast meat, equipment and hands. Improvements and innovations at the poultry processing plant may effectively reduce final production contamination with *Listeria*.

INTRODUCTION

*Listeria monocytogenes* is of major concern to the meat industry and several outbreaks of listeriosis have in recent times occurred in Europe (Salvat et al. 1995; Dorozynski 2000). The pathogen has been found regularly in poultry products and environments where meat is processed. It is capable of...
surviving in these environments for long periods (Salvat et al. 1995) and frequencies of 41–84% have been reported from broiler carcasses and raw chicken products (Lawrence and Gilmour 1994; Franco et al. 1995; Uyttendaele et al. 1999).

Although present in the environment and on food raw materials, one of the major sources of *L. monocytogenes* in food products is the processing environment itself. *Listeria monocytogenes* can colonize the processing environment, utensils, brines, etc. (Lawrence and Gilmour 1995; Giovannacci et al. 1999; Rorvik et al. 2000; Fonnesbech Vogel et al. 2001a). This colonization, as well as job rotation of staff among departments, have been identified as primary mechanisms for contamination of the final products in some processing lines. In most cases, the contamination of the final product is believed to have occurred during processing because the strains found in the incoming raw materials are different from the strains found in the final product (Autio et al. 1999; Fonnesbech Vogel et al. 2001a). In other cases, when the strains of *L. monocytogenes* contaminating the raw materials were indistinguishable from those isolated from the final product, the contaminated incoming raw materials are considered to be the source of continuing contamination (Lawrence and Gilmour 1995; Giovannacci et al. 1999; Fonnesbech Vogel et al. 2001a).

Raw broilers are cooked before consumption, but there is a risk of cross-contamination with other foodstuffs in the consumer’s kitchen. *Listeria monocytogenes* is able to grow and form biofilms on various food-processing surfaces, enhancing survival (Miettinen et al. 2001). Improper cleaning and disinfecting of machines in poultry abattoirs may lead to contamination of the poultry carcasses.

The focus of this study was to evaluate the incidence and level of *Listeria* spp. and *L. monocytogenes* contamination at one section of broiler meat processing located in the abattoir. Furthermore, we aimed to discover where *L. monocytogenes* contamination occurs in the processing line of chicken breast meat. The contaminating stages need to be identified so that they can undergo special cleaning and disinfection.

**MATERIALS AND METHODS**

**Sampling Procedure**

A state inspected poultry processing plant cooperated in this study. Conventional slaughtering and processing techniques are used in this plant that processes approximately 12,000 carcasses daily. The poultry was scalded at 55°C for 90 s. Potable water was used in the scalding tank and feather pickers.
Prechiller and chiller waters contained up to 100 p.p.m. chlorine. Twenty percent of the whole chicken was placed in an ice chest and transferred to the cutting room. The pieces were classified and packaged in the same room. The temperature of the processing environment ranged between 27 and 32°C in the bleeding, scalding and defeathering areas and 12–15°C in the evisceration, cutting and packaging rooms. The equipment and environment were cleaned at midday and at the end of the day using alkaline soap and a 34.4% (w/v) quaternary ammonium compound.

Slaughterhouse sampling sites included: 10 samples of liver taken immediately after coming out of the giblet chiller and 10 samples of chicken breast meat taken directly from the package at the end of the processing line, 10 samples from mechanical saws, 10 samples from the stainless steel cutting table, 10 samples from bare hands and 10 samples from hands with gloves worn by the food handler.

Saws and tables were sampled by wetting sterile polyurethane sponges (5 × 2 × 5 cm) in PBS (pH 7) with 0.5% sodium thiosulfate and 0.1% tween to neutralize compounds used for cleaning. For the table, the sampling was performed on an area of 0.6 × 1.0 m and for saws in the cutting area (0.03 × 0.2 m). The sponges were placed into sterile plastic bags that contained 100 mL of *Listeria* Enrichment University of Vermont Medium (UVM). Samples of worker hands and gloves were obtained using sterile polyurethane sponges too. All samples collected at the processing plant were placed in an insulated cold box filled with ice and brought to the laboratory for analysis within 6 h of collection (Messer et al. 1992). Decimal dilutions from homogenate were prepared in 0.1% (w/v) peptone water.

Polyurethane sponges were acquired in local supermarkets and tested for inhibition properties (Daley et al. 1995).

In the laboratory, portions of 25 g of chicken breast meat and liver were obtained in laminar flow hood, placed in an aluminum cup containing 225 mL of *Listeria* Enrichment Broth (LEB) and then homogenized using a homogenizer for 2 min. Decimal dilutions from homogenate were prepared in 0.1% (w/v) peptone water.

**Isolation and Enumeration of *Listeria* spp.**

Counting of *Listeria* spp. was carried out using the Most Probable Number (MPN) technique. For this, all bags and bottles containing the samples in UVM and LEB were incubated aerobically at 30°C for 24 h. After this, the contents were mixed very well and a volume of 10 mL from the first dilution (10⁻¹) of foods and second dilution (10⁻²) of equipment, hands and gloves was transferred to three sterile empty tubes and a volume of 1 mL of the same dilution was transferred to three other sterile tubes containing 9 mL of LEB
in order to obtain the second and third dilutions, respectively. A volume of 1 mL from the $10^{-2}$ and $10^{-3}$ dilutions from food samples and 1 mL of the $10^{-3}$ and $10^{-4}$ dilutions from equipment, hands and gloves samples in peptone water was transferred to another three tubes series containing 9 mL of LEB, to obtain the dilutions $10^{-3}$ and $10^{-4}$ (foods) and $10^{-4}$ and $10^{-5}$ (equipment, hands and gloves). The inoculated tubes were incubated at 30°C for 24 h and then an aliquot of 0.1 mL from each tube was transferred to other tubes containing 10 mL of Fraser broth and incubated at 35°C for 48 h. To confirm the MNP of *Listeria* spp. 0.01 mL of dark Fraser was streaked onto Modified Oxford medium (MOX). At least five typical dark esculin-positive colonies from each plate served to identify *Listeria* spp. using the same procedure as that used for its detection (Donnelly *et al.* 1992). MPN estimation was obtained from the number of tubes with *Listeria* confirmed (after streaking on MOX and biochemical and CAMP tests, “true” MPN) and from the number of dark Fraser broth tubes (“predictive” MPN).

Serotyping of isolates was carried out by agglutination reactions with absorbed rabbit antisera (somatic and flagellar) as described by Seeliger and Hohne (1979). The antisera were obtained from Department of Bacteriology, Oswaldo Cruz Institute, RJ.

A table was used to calculate the MPN of *Listeria* spp. (AOAC 1996).

All microbiological media were from Difco Laboratories, Detroit, Michigan.

**RESULTS AND DISCUSSION**

**Prevalence and Most Probable Number of *Listeria* spp. in Chicken Breast Meat and Chicken Liver**

The data in Tables 1 and 2 show the prevalence and the MPN/g of species of *Listeria* isolated from chicken breast meat and chicken liver, respectively.

A total of 40% of chicken breast samples were contaminated by *L. monocytogenes* serotype 1/2b (Table 1). All nonhaemolytic isolates were identified as *L. innocua* serotype 6a and *L. grayi*. MPN/g of *L. monocytogenes* were found within the range <3–9.1 (Table 2).

Inoue *et al.* (2000) also isolated *L. monocytogenes* from 37.0% of 46 minced chicken samples in Japan and serotypes 1/2a and 1/2b were isolated from all the food groups investigated. According to the authors, the MPN values were higher than 100/g in five (10.9%) minced chicken samples. In this present work despite that the frequency of *L. monocytogenes* isolation has been similar to that presented by Inoue *et al.* (2000), the MPN value observed was lower.
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Pini and Gilbert (1988) isolated *L. monocytogenes* serotypes 1/2, 3a, 3b, 3c, 4b, 4d and nontypable from 60% of raw chicken samples (66% from fresh and 54% from frozen birds), a percentage higher than that observed in our study. Other *Listeria* spp., including *L. welshimeri*, *L. seeligeri* and *L. innocua*, were isolated from 28% of chickens by the authors. Samples of chicken liver investigated in this work were not contaminated by *L. monocytogenes*, but *L. innocua* serotype 6a and *L. grayi* were detected.

According to Engelbrecht *et al.* (1996), the pathogenesis of listeriosis involves sequential expression of internalin A and B genes that induce phagocytosis of the bacteria by intestinal epithelial and liver cells, respectively. So livers could be an important source of pathogenic *L. monocytogenes* to humans. Also, patients with listeriosis showed high antibody levels against internalin from *Listeria*.

Although the occurrence of foodborne listeriosis has not been recognized in Brazil, it has widely been recognized in other countries in association with ingestion of various *L. monocytogenes*-contaminated foods, e.g., seafood, vegetables, dairy products, meat and poultry products (Farber and Peterkin 1991; Ericsson *et al.* 1997; Brett *et al.* 1998; Goulet *et al.* 1998). In most cases, where quantitative determinations were performed, causative foods were contaminated with levels as high as ≥10⁴ CFU/g (Farber and Peterkin 1991; Ericsson *et al.* 1997; Goulet *et al.* 1998). Based on epidemiological investigations and food surveys for *L. monocytogenes* contamination, levels lower than 100 *L monocytogenes* per g food have been assumed as safe (ICMSF 1994).

### TABLE 1.

**OCCURRENCE OF *LISTERIA* SPP. IN SAMPLES OF CHICKEN BREAST MEAT AND LIVER, SURFACES OF SAWS AND TABLE, HANDS AND GLOVES OF FOOD HANDLERS**

<table>
<thead>
<tr>
<th>Listeria species</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Breast</td>
</tr>
<tr>
<td></td>
<td>P/T (%)</td>
</tr>
<tr>
<td><em>Listeria</em> spp.</td>
<td>4/10</td>
</tr>
<tr>
<td></td>
<td>(40)</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>4/10</td>
</tr>
<tr>
<td></td>
<td>(40)</td>
</tr>
<tr>
<td><em>L. innocua</em></td>
<td>2/10</td>
</tr>
<tr>
<td></td>
<td>(20)</td>
</tr>
<tr>
<td><em>L. grayi</em></td>
<td>1/10</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
</tr>
</tbody>
</table>

P, positive samples; T, total of samples analyzed.
Listeria monocytogenes can be recovered from the working environment where meat, contaminated with the organism, is processed. Thus, cross-contamination of cooked meat, raw vegetables and salads with L. monocytogenes from raw chicken handled in the kitchen or in another food-processing environment is a possibility.

**Prevalence and Most Probable Number of Listeria spp. on Saws and Tables**

In this study, Listeria spp. were isolated in 50% of samples of the surface of mechanic saws (Table 1). Listeria monocytogenes serotypes 1/2a, 1/2b, 4b and 1/2c, and L. innocua serotype 6a were isolated in three of the five (60%) samples contaminated. Listeria grayi were detected in all samples contaminated. Eight samples (80%) of the surface of the cutting table were contaminated with L. monocytogenes (Table 1). High levels of L. monocytogenes were present on these surfaces, e.g., for saws <30–2400 MPN/equipment (<0.005–0.4 MPN/cm²) and for table, <30–11,000 MPN/equipment (<0.0005–0.183 MPN/cm²) (Table 2).

Autio et al. (2000) also investigated 73 environment samples from 10 abattoirs and isolated L. monocytogenes from 2 samples from a saw and 1 sample from a drain, a door and a table in the cutting room. According to the authors, the carcass samples were contaminated with L. monocytogenes in two abattoirs where the mechanical saws were used to cut the breasts. In this present study, L. monocytogenes was isolated from saws and tables at a higher frequency than that reported by Autio et al. (2000).

The presence in high numbers of Listeria spp. and L. monocytogenes in table and saws indicated that the disinfection procedures used at the poultry processing plant investigated in this study were ineffective.

According to Ryser and Marth (1999), industries in various countries that investigate the presence of Listeria and other pathogenic microorganisms in their processing plants and final products do not pass on the information to the scientific community. The authors considered that this is probably attributed to fear of negative publicity and risk of financial loss. Therefore, the amount of data published on the incidence of Listeria at industrial installations is small.

**Prevalence and Most Probable Number of Listeria spp. on Bare Hands and Hands with Gloves of Food Handlers**

In the samples of the bare hands of food handlers 6 of the 10 (60%) samples presented Listeria, of which 2 (33.33%) harbored L. monocytogenes serotype 4b. Listeria innocua 6a, 6b and nontypable and L. grayi were isolated too. On hands with gloves only L. innocua and L. grayi were
TABLE 2.
MOST PROBABLE NUMBER OF *LISTERIA* SPECIES IN SAMPLES OF CHICKEN BREAST MEAT AND LIVER, SURFACES OF SAWs AND TABLE, HANDS AND GLOVES OF FOOD HANDLER

<table>
<thead>
<tr>
<th>Samples</th>
<th><em>Listeria</em> spp.</th>
<th><em>L. monocytogenes</em></th>
<th><em>L. innocua</em></th>
<th><em>L. grayi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>&lt;3.0–23.0 (MPN/g)</td>
<td>&lt;3.0–9.1 (MPN/g)</td>
<td>&lt;3.0–3.6 (MPN/g)</td>
<td>&lt;3.0–3.6 (MPN/g)</td>
</tr>
<tr>
<td>Liver</td>
<td>&lt;3.0–9.0 (MPN/g)</td>
<td>&lt;3.0 (MPN/g)</td>
<td>&lt;3.0–3.6 (MPN/g)</td>
<td>&lt;3.0–9.1 (MPN/g)</td>
</tr>
<tr>
<td>Gloves</td>
<td>&lt;30–36 (MPN/glove)</td>
<td>&lt;30 (MPN/glove)</td>
<td>&lt;30–36 (MPN/glove)</td>
<td>&lt;30–30 (MPN/glove)</td>
</tr>
<tr>
<td>Saw</td>
<td>&lt;30–2400 (MPN/equip)</td>
<td>&lt;30–2400 (MPN/equip)</td>
<td>&lt;30–390 (MPN/equip)</td>
<td>&lt;30–150 (MPN/equip)</td>
</tr>
<tr>
<td>Table</td>
<td>91 to &gt;11,000 (MPN/equip)</td>
<td>&lt;30–11,000 (MPN/equip)</td>
<td>&lt;30 to &gt;11,000 (MPN/equip)</td>
<td>&lt;30–2100 (MPN/equip)</td>
</tr>
</tbody>
</table>
isolated. The levels of *Listeria* on hands and gloves were low (<110 MPN/hand) (Table 2).

Genigeorgis *et al.* (1989) also analyzed swabs from gloves and the workers’ hands, and found that up to 59% of the people who worked at the end of the production line were carriers of *Listeria*. In the 1990, Genigeorgis *et al.* (1990) carried out a study at a turkey processing plant and found that samples of hands and gloves of food handlers, who hang up the carcasses after the chilling, cutting the carcasses and packaging, were contaminated by *Listeria* spp. at a frequency of 16.7%, 33.3% and 40.0%, respectively. Kerr *et al.* (1993) investigated 99 workers’ hands at 44 establishments and found that 12 (12%) food workers carried *Listeria* spp. and 7 (7%) carried *L. monocytogenes*. Our results indicate that the prevalence of *Listeria* spp. on the hands is very similar to that presented by Genigeorgis *et al.* (1989). However, the results concerning the prevalence of *Listeria* spp. and *L. monocytogenes* on these surfaces were higher than that reported by Kerr *et al.* (1993).

The chicken breast meat is usually eaten after being cooked, and therefore this food may be of low risk. However, the opportunities of cross-contamination with other foodstuffs in the consumer’s kitchen should be considered, so the food industry should not ignore Good Manufacturing Practices (GMP) standards. Additionally an effective and properly applied sanitation program should be strictly followed to prevent *L. monocytogenes* contamination.

**ACKNOWLEDGMENTS**

We are grateful to Dr. Cristhiane Moura Falavina dos Reis and Ernesto Hofer (Brazil) for assistance in performing the serological analysis of *Listeria* species. This study was supported by CAPES, Brazil.

**REFERENCES**


