Listeria monocytogenes in Brazilian Foods: Occurrence, Risks to Human Health and Their Prevention

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Abstract

Listeria monocytogenes is a foodborne pathogen which occurs mainly in ready-to-eat food products, especially in artisanal products manufactured from raw milk such as some types of cheese, meat products and leafy vegetables. L. monocytogenes requires special attention in the food industry because of its ability to survive under adverse conditions and form biofilms on different surfaces in food processing environments. The potential for product contamination by L. monocytogenes strains in the industrial environment emphasizes the importance of preventive measures in the food industry. This review presents an overview on the main characteristics, pathogenicity and occurrence data of L. monocytogenes in Brazilian foods. The main prevention measures to avoid contamination by L. monocytogenes in foods are also highlighted, especially the adoption of quality assurance programs by the food industry.

Introduction

The genus Listeria comprises several species of Gram positive bacteria which occur widely in nature, being frequently found in soil, decomposing plants, sewage, silage, dust and water.¹ These species include L. monocytogenes, L. ivanovii, L. innocua, L. welshimeri, L. seeligeri, L. grayi, L. marthii and L. rocourt.¹ Due to its ubiquitous nature, L. monocytogenes and other species can often contaminate food products at various points in the production and processing chain. L. monocytogenes and other species of the genus have already been
found in several unexpected urban environments, which were identified as potential reservoirs, such as sidewalks, doorknobs, signaling equipment and grab bars. While *L. seeligeri* and *L. welshimeri* have been associated with natural environments, *L. innocua* and *L. monocytogenes* are often identified in urban environments. However, only *L. monocytogenes* and *L. ivanovii* are considered pathogenic for humans and animals, with *L. ivanovii* causing milder listeriosis, while *L. monocytogenes* is the causative agent of serious infections such as meningitis, encephalitis, and sepsis.

*L. monocytogenes* strains can be differentiated on the basis of certain antigens expressed on the surface of the bacterium. These variations are produced by distinct surface structures containing lipoteichoic acid, membrane proteins and extracellular organelles such as flagella and fimbriae. These differences can be identified by serology, thus *L. monocytogenes* is classified into thirteen serotypes based on somatic and flagellar antigens (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4b, 4c, 4d, 4e and 7). It should be noted, however, that the majority of cases of human listeriosis are mainly associated with serovars 1/2a, 1/2b and 4b, with 4b being the most frequently related to cases of human listeriosis.

*L. monocytogenes* was firstly identified in the early twentieth century, although its definitive classification as a bacterium occurred only in 1940. Since then, *L. monocytogenes* has been considered as an opportunistic pathogen to humans, because its development is favored in immunocompromised, pregnant, and newborn individuals. Listeriosis is a result of the ingestion of foods contaminated with *L. monocytogenes*, mainly products that were kept refrigerated for extended periods and consumed without previous heating, such as dairy products including milk, cheese and butter and other ready-to-eat foods, like sausages, pâté, fish and minimally processed vegetables.

There are two forms of clinical manifestation of the disease: invasive and noninvasive. Invasive listeriosis does not cause gastrointestinal symptoms in humans, since the cells that colonize the intestine are phagocytosed by macrophages. After this event, *L. monocytogenes* cells multiply and spread throughout several organs in the body, causing various forms of infections such as meningitis and encephalitis in the central nervous system, endocarditis (heart), peritonitis (abdomen), pneumonia (lungs) and osteomyelitis (bones).

Although most immunocompetent individuals outgrow the initial attack and eliminate *L. monocytogenes* by the feces, people at high risk for the microorganism including immunocompromised, neonates, pregnant women and elderly, can be affected by the invasive form of disease. Clinical signs in pregnant women include abortion, premature birth, meningitis, and neonatal sepsis. In outbreaks of invasive listeriosis, the most related food products are ready-to-eat foods such as cheese, meat products and leafy vegetables. The noninvasive form is characterized by gastroenteritis, a milder form of listeriosis, which does not appear to affect any specific population groups.

In Brazil, human listeriosis is underdiagnosed and underreported, and there are no records of foodborne cases despite *L. monocytogenes* often being isolated in several products, especially dairy products. The potential risk of foodborne *L. monocytogenes* stress the need of surveillance practices as well the adoption of prevention procedures especially in companies that industrialize, manipulate, prepare or serve ready-to-eat foods. The objective of the present work was to review the data reported on the occurrence of *L. monocytogenes* in the main risk food products commercialized in Brazil, to discuss the risks associated with contaminated foods for the human health, and to describe the main strategies for the prevention of contamination by this pathogen in food industries.

**Characteristics of Listeria monocytogenes**

The species from the genus *Listeria* are small, non-sporogenic Gram positive rods with 0.5 μm x 1-2 μm, facultative anaerobes, and with motility by peritrichal flagella at temperatures between 20 °C and 25 °C. They can grow at a wide temperature range (1 to 45 °C), with optimum growth between 30 and 37°C. The optimal water activity for their development occur in the range of 0.92 to 0.99. Importantly, *L. monocytogenes* is a pathogenic bacterium that may be present in the food processing environment, which makes environmental components significant sources of food contamination. The main reservoir of *L. monocytogenes* is the soil, followed by sludge,
fodder and water. Some studies suggest that 1-10% of humans can carry L. monocytogenes in the intestine without presenting symptoms of listeriosis, and that this bacterium has been found in at least 37 mammal species (domestic and wild), 17 species of birds and some species of fish and seafood.

The dissemination of L. monocytogenes can occur through feces of animals and human intestinal carriers of the microorganism. Factors that classify this microorganism as relevant for public health and for the food industry are its metabolic and physiological characteristics, such as the ability to multiply in refrigeration temperatures, ability to survive in high concentrations of sodium chloride and nitrates and in a wide range of pH (between 4.3 and 9.6), besides its ability to form biofilms that are difficult to remove. Although L. monocytogenes does not form spores, it resists well the deleterious effects of freezing and drying, as well as high concentrations of NaCl. In this context, the increased production of refrigerated, ready-to-eat and minimally processed products aiming to preserve the true flavor of the food, may also contribute for the emergence of niches with proliferation of L. monocytogenes. For this reason, food products that can support the development of L. monocytogenes should be kept under refrigeration at temperatures below 4.4 °C, to avoid significant bacterial growth during storage.

L. monocytogenes has three phylogenetic lineages, namely types I, II and III.20 Lineage type I, characterized by the serotypes 1/2b, 3b, 4b, 4d and 4e, is highlighted by the predominance of serotypes related to the occurrence of listeriosis in humans.20 Lineage type II, composed by serotypes 1/2a, 3a, 1/2c and 3c, comprise the main serotypes isolated from food matrices. Finally, the lineage type III include the serotypes 4a, 4c and 7, which are considered less harmful to human health, but commonly related to the occurrence of listeriosis in animals. Subtyping methods have allowed researchers to verify that some strains of L. monocytogenes can colonize food processing environments, and remain as residents for months or even years, in the absence of correct sanitization procedures. Tompkin (2002) summarized the outcomes from studies on the persistence of L. monocytogenes in food industries, especially in dairy factories. In Sweden, a strain of L. monocytogenes (serotype 3b) persisted for seven years in a Blue Veined cheese processing plant. In Switzerland, a serotype 4b strain remained for 11 months in the goat cheese processing environments. In another study conducted in a cheese processing plant from United Kingdom, it was observed that a serotype 4b strain persisted for four years. These data demonstrated incontestable examples of resistance of L. monocytogenes in food processing environments.

Pathogenesis of Infections Caused by Listeria monocytogenes

The first diagnosis of listeriosis in humans was made in 1924 by E. Murray, in a soldier affected by meningitis. Although initially designated by Murray as Bacterium monocytogenes, the microorganism was identified only 2 years after its first occurrence, through the isolation of Gram positive rods, which were not previously classified in any genus, from blood samples of laboratory animals that suffered sudden death. In 1940, Harvey Pirie, while working with food, human blood, and environmental samples, included B. monocytogenes in the genus Listeria. Since its first description, L. monocytogenes has been considered as a pathogenic organism in more than 50 species, among them, humans and many mammals, wild birds, fish and crustaceans. Although listeriosis was initially treated as an animal disease, and considered rare and sporadic in humans, L. monocytogenes was recognized as a foodborne pathogen for humans only in the 1980s, when the investigation of a human listeriosis outbreak linked to the consumption of various foods contaminated with the bacterium. Subsequently, the bacterium was included in the list of food pathogens in 1981, when another listeriosis outbreak occurred in Canada due to the consumption of contaminated coleslaw. The first genome sequencing of L. monocytogenes lineage EGD serotype 1/2a was described in the scientific literature in 2001. Since L. monocytogenes is an intracellular pathogen, it is capable of penetrating and multiplying in the cytoplasm of the host cell, as well as protecting itself from the immune system, which brings great interest in research related to cell biology. In the process of pathogenesis, factors such as alkalinization of the stomach by antacids, previous history of co-morbidities of the gastrointestinal tract including
gastrointestinal infections and previous surgeries of ulcers may favor infection in individuals already colonized with *L. monocytogenes*.\(^9\)

The main determinant factors for the occurrence of listeriosis outbreaks are the lack of adequate heat treatment to destroy the microorganism in the food, the composition of the food that favors the multiplication of *L. monocytogenes* and the consumption of these foods by individuals belonging to the risk group.\(^17\) Consumers considered in risk groups are the elderly over 70, alcoholics, diabetics, anemic, pregnant, newborns, and immunosuppressed individuals such as cancer patients, patients undergoing hemodialysis, and extended therapies.\(^8\) The incubation period of listeriosis varies from hours to weeks\(^29\), and the infective dose of *L. monocytogenes* depends on the strain in question and the immune system of the individual exposed to this bacterium. Some studies indicate that counts below 102 colony forming units (CFU)/g of food are not infectious, although this possibility is not excluded.\(^16\) In general, it is assumed that infective doses of less than 1,000 cells can cause disease in susceptible populations.\(^17\)

The infectious process appears to be triggered by an association between the microorganism and the plasma membrane of epithelial cells of the microvilli of the host intestinal tract.\(^12\) After this association, the microorganism is internalized in the cell by phagocytosis, remaining in the vacuole for a short period of time until the lysis of the phagosome membrane. After releasing the bacterium into the cytoplasm of the host cell, it multiplies rapidly and induces filament polymerization of actin from the host cell, forming long tails at one end of the bacterial cell.\(^11,12\) The actin filaments favor the displacement of the bacterium in the cytoplasm, allowing the invasion of adjacent cells giving rise to new cycles of infection. The infectious process requires the production and action of various bacterial proteins on the components of the host cell to carry out its intracellular life cycle, and all proteins involved in the cycle of infection are encoded by specific virulence genes.\(^8\)

As mentioned previously, two forms of clinical manifestation of listeriosis are described: invasive and gastrointestinal (non-invasive).\(^30\) The first is characterized by severity, presenting high mortality rates (20-30%), mainly in risk groups.\(^12\) Studies demonstrated that pregnant women are 12 times more likely to be affected by listeriosis than the general population.\(^26\) In general, infected pregnant women exhibit only mild symptoms. However, there is an increasing probability of abortion and premature labor due to the invasion of the placenta by the bacterium reaching the fetus, which does not yet have an immune system capable of defending itself from the agent.\(^30\) Additionally, neonatal infections can occur during labor through the birth canal.\(^25\)

The fetal mortality rate is 16-45%, and 20% of pregnant women with listeriosis suffer miscarriage while 63% of these women have babies with neonatal infection.\(^26\) Multiparous women are more likely to develop listeriosis in the gestational period when compared to nulliparous women (first gestation).\(^27\)

Other complications following exposure of *L. monocytogenes* include encephalitis, meningitis, endocarditis, granulomatous lesions in the liver and other organs, internal or external abscesses, and pustular skin lesions.\(^3\) *L. monocytogenes* is considered an important cause of meningitis, although rare, but with a mortality rate of approximately 30%.\(^3\) Noninvasive listeriosis, on the other hand, is not associated with deaths and causes mild, flu-like infections in healthy individuals, and may progress to outbreaks of febrile gastroenteritis, many of which are not identified as listeriosis.\(^25\)

In Brazil, between 2007 and 2017, 134,046 individuals were sick with foodborne diseases; 19,394 were hospitalized, and there were 127 registered deaths.\(^32\) The available data indicated that mostly of foodborne outbreaks were directly related to the consumption of animal origin food, such as dairy products, beef, pork, poultry and fishery products.\(^32\) However, outbreaks or sporadic cases of food-related listeriosis have not yet been reported, although the occurrence of *L. monocytogenes* has been reported regularly in the last decades in various types of food, especially in minimally processed cheese and vegetables.\(^33,34\) Listeriosis is not a notifiable disease in the country, and despite few official records of occurrence, it represents a major concern for public health authorities. The absence of cases of listeriosis has been attributed in part to
its misdiagnosis with other diseases, such as acute gastroenterocolitis, especially in relation to the noninvasive form.9

Occurrence of L. monocytogenes in Brazilian Foods
The available data on the incidence of L. monocytogenes in foods indicate that cheeses present the highest risk of contamination, although meat products and leafy vegetables consumed in natura may also be important in the epidemiology of listeriosis.33,34,35 Barancelli et al.,9 reviewed the published data on the occurrence of L. monocytogenes in dairy products in Brazil from 1991 to 2009, and found a wide range of occurrence (zero to 41%) of the microorganism in cheese. The authors considered that this variation can be attributed to the use of different methods for the detection of bacteria, differences in quality standards of cheese and the use of raw or pasteurized milk as raw material. There are only a few studies published after 2010 on the occurrence of L. monocytogenes in Brazilian dairy products. In a survey conducted in 2011 in three dairy plants from the State of São Paulo, L. monocytogenes was detected in two of them, at rates of 9.1% and 12.5% of environmental samples evaluated.14 No isolation of L. monocytogenes was observed in samples of raw milk from silos, pasteurized milk, water, and Minas Frescal cheese from the three monitored plants. However, the authors detected L. monocytogenes in Prato cheese and brine samples from one of the dairy plants, which highlights the importance of environmental monitoring programs and control strategies in cheese production plants.14 Recently, another study analyzed 164 cheese samples produced in 2017 by five dairy plants from the states of São Paulo and Goiás, and did not find any positive for L. monocytogenes, indicating that the contamination rate in dairy products decreased significantly when compared to previous studies.36 However, the authors observed that, out of 16 samples of Mozzarella cheese available for sale in retail in sliced form or in pieces, three (0.7%) tested positive for L. monocytogenes, hence evidencing the occurrence of failures in hygiene procedures in the commercialization of the product.36

Cheese is a ready-to-eat food of great popularity in Brazil, being considered one of the most consumed dairy products worldwide. In addition to the diversity of flavors, cheese is a rich source of calcium, phosphorus, and protein in the diet.37 Cheese that goes through the fungus maturation process present an increase of pH, which decreases L. monocytogenes proliferation, whereas cheese with low water activity and lower pH are also unfavorable for the growth of this microorganism.37 Most pathogenic bacteria, including L. monocytogenes, does not find favorable environment for multiplication in hard (moisture ≤ 39%) and semi-hard (moisture between 39-50%) cheese.38 Consequently, most outbreaks of listeriosis have been associated with the consumption of soft cheese (moisture ≥50%) produced under inadequate hygienic conditions during handling at the production process, or when cheese are produced with raw milk.38 In Brazil, fresh cheeses are the most consumed, mainly Minas Frescal, which presents high moisture content, pH values around 4.9-5.3, humidity levels (55-58%), and salt concentrations between 1.4 and 1.6%.36 In addition, Minas Frescal cheese is subjected to hand manipulation during manufacturing, thus facilitating the contamination, survival and multiplication of deteriorating and pathogenic bacteria, such as L. monocytogenes.36 Additionally, in the Northeast region of Brazil, artisanal cheeses such as curd and butter-type cheese are the most consumed by the general population.39 The production of artisanal curd cheeses often use a large amount of raw milk, leading to microbiological problems, such as contamination with pathogenic bacteria.39 The occurrence of L. monocytogenes in raw milk in Brazil was found to range from 0 to 37%.9 However, even cheese made with pasteurized milk can be contaminated by the pathogen, since the industrial environment can serve as an important source of contamination of L. monocytogenes.5,33 In industrial facilities, L. monocytogenes can be found in drains, mats, refrigerators, air filters, walls, cleaning utensils and other environments that remain wet for long periods, failing the proliferation and maintenance of this bacteria for long periods due to its ability to form biofilms on surfaces.14 Biofilms are structured bacterial communities surrounded in their own polymeric extracellular matrix adhered to a biotic or abiotic surface.18,23
The occurrence data of *L. monocytogenes* in other food products such as minimally processed meat and vegetable products marketed in several Brazilian states from 2001 to date are presented in Table 1. Kasnowski\(^\text{40}\) analyzed 30 samples of bovine meat (rump steak) marketed in the city of Rio de Janeiro found that a greater number of isolates (16 colonies, 57.1%) was obtained in ground meat. The main species identified were *L. innocua* and *L. monocytogenes* belonging to serogroup 4b, with great resistance to antimicrobial agents.\(^\text{40}\) However, a lower percentage of positive samples (6.7%) was observed by Mantilla \textit{et al.}\(^\text{35}\) in ground meat commercialized in the state of Rio de Janeiro. In a survey on the incidence of *L. monocytogenes* in retailed salami and cooked ham purchased in São Paulo city, the pathogen was detected in 6.2% and 0.8% of samples analyzed, respectively.\(^\text{41}\) Among the strains found in both analyzed products, the serotypes identified and their respective percentages were: 4b (37.5%), 1/2b (25%), 3b (25%) and 1/2c (12.5%). The authors stated that the risk of listeriosis due to the consumption of salami is greater than that associated with the consumption of ham.\(^\text{41}\) Another study conducted in the state of Ceará indicated a high incidence (42.5%) of *L. monocytogenes* in commercially available ham samples.\(^\text{42}\) More recent surveys indicated incidences of 6.0-8.2% in bovine carcasses and sausages commercially available in several Brazilian states.\(^\text{43,44}\) Regarding leafy vegetables, the incidence of *L. monocytogenes* reported in São Paulo state was lower than those described for cheese or meat products, varying from 0.6 to 5.3%.\(^\text{45,46,48,49,50,51}\) Additionally, no *L. monocytogenes* was detected in minimally processed vegetables such as cress, lettuce, carrot, spinach, green cabbage commercialized in Fortaleza, Ceará, although other species of the genus *Listeria* were found.\(^\text{47}\) In the state of Bahia, *L. monocytogenes* was detected in 3.0% of analyzed samples of raw, frozen and ready-to-eat vegetables. Results from the above mentioned studies indicate

<table>
<thead>
<tr>
<th>State</th>
<th>Type of food</th>
<th>Year</th>
<th>N*</th>
<th>n (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meat products:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Rio de Janeiro</td>
<td>Bovine meat</td>
<td>2004</td>
<td>30</td>
<td>16 (53.3)</td>
<td>40</td>
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<tr>
<td>Rio de Janeiro</td>
<td>Bovine ground meat</td>
<td>2004</td>
<td>30</td>
<td>2 (6.7)</td>
<td>35</td>
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<tr>
<td>São Paulo</td>
<td>Salami</td>
<td>2009</td>
<td>130</td>
<td>8 (6.2)</td>
<td>41</td>
</tr>
<tr>
<td>São Paulo</td>
<td>Cooked pork ham</td>
<td>2009</td>
<td>130</td>
<td>1 (0.8)</td>
<td>41</td>
</tr>
<tr>
<td>Ceará</td>
<td>Cooked pork ham</td>
<td>2011</td>
<td>40</td>
<td>17 (42.5)</td>
<td>42</td>
</tr>
<tr>
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<td>2017</td>
<td>200</td>
<td>12 (6.0)</td>
<td>44</td>
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<tr>
<td>Several states a</td>
<td>Sausage</td>
<td>2018</td>
<td>98</td>
<td>8 (8.2)</td>
<td>43</td>
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<tr>
<td><strong>Minimally processed vegetables:</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>São Paulo</td>
<td>Leafy vegetables</td>
<td>2001</td>
<td>150</td>
<td>8 (5.3)</td>
<td>45</td>
</tr>
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<td>2007</td>
<td>181</td>
<td>1 (0.5)</td>
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<tr>
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<tr>
<td>São Paulo</td>
<td>Leafy vegetables</td>
<td>2011</td>
<td>162</td>
<td>2 (1.2)</td>
<td>48</td>
</tr>
<tr>
<td>São Paulo</td>
<td>Leafy vegetables</td>
<td>2012</td>
<td>512</td>
<td>16 (3.1)</td>
<td>49</td>
</tr>
<tr>
<td>São Paulo</td>
<td>Leafy vegetables</td>
<td>2012</td>
<td>172</td>
<td>2 (1.2)</td>
<td>50</td>
</tr>
<tr>
<td>Bahia</td>
<td>Raw, frozen and ready-to-eat vegetables</td>
<td>2016</td>
<td>132</td>
<td>4 (3.0)</td>
<td>51</td>
</tr>
</tbody>
</table>

\(^*\) States of Rio Grande do Sul, São Paulo, Minas Gerais, Goiás and Pernambuco.

N* = number of samples analyzed.
n (%) = number and percentage of samples positive for *L. monocytogenes*. 

Table 1: Occurrence of *L. monocytogenes* in meat products and minimally processed vegetables marketed in Brazil
possible hygiene failures during manufacture of ready-to-eat food products in Brazil. Moreover, the occurrence rates of *L. monocytogenes* as described in meat products and leafy vegetables in Brazil may have a significant health risks to consumers. In this context, the successful implementation of a quality programs is essential for reducing the probability of product contamination in food industries, and to comply with tolerance limits set by food safety authorities.\(^{52}\)

Globally, there are strict health protection policies related to the presence of *L. monocytogenes* in food products. The United States has established zero tolerance for this bacterium in ready-to-eat foods, including dairy products, based on the probability that the minimum infecting dose may be low.\(^{17}\) In other countries including Canada, foods are categorized in groups according to the conditions they provide for the growth of the microorganism, as well as their shelf life and storage conditions. Thus, the tolerable limits for *L. monocytogenes* in Canada vary from absence in 50 g up to <100 CFU/g in the food.\(^{52}\) The European legislation establishes the following guidelines through EU Regulation Reg. 2073/2005: 1) Food processors must ensure the absence of the pathogen in the case of ready-to-eat foods intended for at-risk groups (pregnant women, the elderly, children and immunocompromised); 2) Food must present a maximum population of 100 CFU/g or mL by the expiration date, when the food allows the multiplication of the bacteria, or when they pass through the quality control industry, in case if they do not offer conditions for the multiplication of the pathogen.\(^{53}\) In Brazil, cheese is the only ready-to-eat food with regulations defined for *L. monocytogenes*, which is defined by absence of the bacteria in 25 g in five sample units collected from the same product batch.\(^{54}\)

**Prevention of L. monocytogenes in the Food Industry**

The presence of *L. monocytogenes* in the industrial environment is of great concern because of the potential product contamination. Considering that *L. monocytogenes* can enter continuously in processing sites, its control has been based on strict hygiene and sanitation procedures.\(^{55}\) For high-risk products, an environmental monitoring program should be adopted, especially on surfaces that come into direct contact with food.\(^{36}\) Whenever a given sample tested positive for *L. monocytogenes* or the genus *Listeria*, rapid and effective actions should be taken to prevent food contamination.\(^{38}\) It is important to note that food safety systems are based on a combination of two other elements, in addition to GMP, such as Sanitation Standard Operating Procedures (SSOP) and Hazard Analysis and Critical Control Points (HACCP).\(^{56}\) GMP and SSOP are prerequisite programs for HACCP implementation. The adoption of HACCP systems has been mandatory in Brazilian food industries since 1993, when the guidelines for its implementation were enforced by the Ministry of Health.\(^{57}\) Subsequently, the Brazilian Ministry of Agriculture determined that HACCP systems should be adopted gradually by the animal-derived food products industries.\(^{57}\)

Prerequisite programs and HACCP are essential in food production plants with a high risk of *L. monocytogenes*, such as high moisture cheese processing plants, to reduce the environmental occurrence of this pathogen and to minimize the risks of contamination of products.\(^{58}\) The HACCP system uses its own concepts, whose terminology includes terms such as hazard, risk, critical control point (CCP), critical limit and corrective action, whose definitions can be widely found in the literature.\(^{58}\) Although the HACCP system is recognized as one of the most effective ways to guarantee food quality and safety, it is observed that its adoption is still restricted and diffused more widely among companies which are motivated by the need to comply with the regulatory standards imposed by other countries and the interest in maintaining access to these markets.\(^{59}\) Thus, the adherence of small and medium-size food industries to the HACCP system is still low, including small-scale cheese factories and establishments that process leafy vegetables in Brazil.\(^{58}\) An important limiting factor for implementation of the HACCP in the food industry is the high cost related to economy of scale, mainly due to the lack of a clear understanding of the benefits brought by the plan, which are considered as limited or intangible.\(^{60}\) A good example of successful implementation of GMP was described in a mozzarella cheese processing plant located in the Southwestern region of the state of Parana, Brazil.\(^{61}\) In this factory, the implementation of GMP was carried out in four steps: diagnosis, report of the diagnosis and road map, corrective
measures and follow-up of GMP implementation. The aerobic microorganisms and total coliforms counts in equipment, as well as total coliforms in the hand of food handlers, significantly decreased after the implementation of GMP. Moreover, GMP implementation changed the overall organization of the cheese plant, and the food handlers’ behavior and knowledge on the quality and safety of mozzarella cheese manufactured. It should be noted that, in the case of L. monocytogenes, quality assurance programs are more important considering that this microorganism is capable of producing biofilms on surfaces of equipment and utensils used in industry, such as glass, metal (stainless steel), rubber and polypropylene, which are difficult to decontaminate, allowing them to persist for long periods in the processing environment. The difficulty in eliminating this microorganism from industries is enhanced by the conditions of humidity, temperature and the presence of organic matter in the processing plants, which together with the pathogen ability to produce biofilms can trigger the colonization of equipment and utensils surfaces. The fact that some listeriosis outbreaks have been associated with the occurrence of L. monocytogenes biofilms in dairy products reinforces the importance of the quality assurance programs, especially the strict compliance of the SSOP at all stages of production of food.

Concluding Remarks
The presence of L. monocytogenes and other species is relatively frequent in several foods in Brazil, especially in cheese, meat products and leafy vegetables. However, there are no records of invasive listeriosis related to the consumption of contaminated food in the country, although the literature demonstrates the occurrence of several outbreaks in other countries. Noninvasive listeriosis is characterized by a disease that is difficult to identify because it presents symptoms similar to those of other diseases, and it is certainly underdiagnosed. In Brazil, the legislation establishes the criterion of absence of L. monocytogenes only in medium, high and very high humidity cheeses. Considering the occurrence data of L. monocytogenes in minimally processed vegetables and ready-to-eat meat products such as salami and ham in Brazil, it is necessary to update Brazilian legislation in order to include tolerance limits for these ready-to-eat products. In addition, the successful implementation of a quality programs is an essential factor in reducing the probability of product contamination in food industries.

Acknowledgements
The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support and fellowship – Grant no. 306304/2017-1.

Funding
This study is financially supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Grant no. 306304/2017-1.

Conflict of Interest
The authors declare that they have no conflicts of interest.

References


