







## *Listeria monocytogenes* and its impact on the safety of food of animal origin

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**ABSTRACT:** This research detected *Listeria monocytogenes* in samples of animal origin and equipment from processing industries using Real-Time PCR and VIDAS LMO 2 techniques. We analyzed 248 samples collected in dairy products, chicken meat, frozen, beef cuts, mechanically deboned chicken meat, fresh giblets, processed meat products and equipment of Brazilian processing plants. The microorganism was detected in 7.3% (18/248) of samples associating the two methodologies, in 6.4% (16/248) by Real-Time PCR and 4.0% (10/248) by the other method. *L. monocytogenes* was detected in 3.1% (3/95) of dairy products, 4.0% (3/74) of chicken meat, 84.6% (11/13) mechanically deboned chicken meat and 8.3% (1/12) processed meat products. The positive samples included mozzarella, fresh Minas cheese, chicken meat cut, mechanically deboned chicken meat, fresh chicken sausage. The bacteria were absent in frozen, beef cuts and equipment food processing industry samples. The presence of the bacteria in cheeses, chicken meats, and sausages indicated that these foods can pose risks to public health and emphasizes the need to implement control measures during processing. Equally worrying is the presence of the microorganism in mechanically deboned chicken meat and in the raw material for countless processed meat products.

**Key words:** animal products, foods, public health, listeriosis, detection methods, Brazilian abattoirs.

## *Listeria monocytogenes* e seu impacto na segurança de alimentos de origem animal

**RESUMO:** Este trabalho teve como objetivo detectar *Listeria monocytogenes* em amostras de produtos de origem animal e em equipamentos de indústrias de processamento utilizando as técnicas de PCR em Tempo Real e VIDAS LMO 2. Foram analisadas 248 amostras, entre laticínios, carnes de frango, gelados comestíveis, cortes bovinos, carne de frango mecanicamente separada, miúdos frescos, produtos cárneos processados e equipamentos em indústrias de alimentos brasileiras. O microrganismo foi detectado em 18/248 (7,3%) amostras associando as duas metodologias, em 16/248 (6,4%) pelo PCR em Tempo Real e em 10/248 (4,0%) pelo outro método. *L. monocytogenes* foi detectada em 3,1% (3/95) de produtos lácteos, 4,0% (3/74) de carnes de frango, 84,6% (11/13) de carne de frango mecanicamente separada e 8,3% (1/12) de produtos cárneos processados. As amostras positivas incluíram os queijos muçarela e Minas frescal, cortes comerciais de frango, carne de frango mecanicamente separada e linguiça frescal de frango. Amostras de gelados comestíveis, de cortes cárneos bovinos e de equipamentos da indústria de processamento de salsichas foram negativas. Houve detecção da bactéria em queijos, carnes de frango e embutidos, o que indica que esses alimentos podem representar risco à Saúde Pública e enfatiza a necessidade de implementação de medidas de controle durante o processamento desses produtos. Igualmente preocupante é a presença do microrganismo em carnes mecanicamente separadas de frangos, matéria-prima de incontáveis produtos cárneos processados.

**Palavras-chave:** produtos de origem animal, alimentos, Saúde Pública, listeriose, métodos de detecção, abatedouros brasileiros.

## INTRODUCTION

Although the genus *Listeria* consists of species *L. aquatic*, *L. booriae*, *L. cornellensis*, *L. denitrificans*, *L. fleischmannii*, *L. floridensis*, *L. grandensis*, *L. grayi*, *L. innocua*, *L. ivanovii*, *L. marthii*, *L. monocytogenes*, *L. murrayi*, *L. newyorkensis*, *L. riparia*, *L. rocourtiae*, *L. seeligeri*, *L. weihenstephanensis*, *L. welshimeri* (PARTE et al., 2020), the human listeriosis can be caused only by *L. monocytogenes* and *L. ivanovii* (QUEREDA et al., 2021).

A pathogen of concern to the food industry, *L. monocytogenes* is often isolated from animal products (PALMA et al., 2020). The illness can manifest itself invasively and non-invasively. The first, more severe, can cause meningitis, septicemia, abortion, meningoencephalitis, and endocarditis, usually affecting patients with deficient immunological conditions, which can lead to death (KALANI et al., 2018; RADOSHEVICH & COSSART, 2018). In the non-invasive form, infections are mild, similar to flu or gastroenteritis, usually not progressing to death (HENKEL et al., 2015). Although, it is a food-

borne disease of low occurrence, human listeriosis is characterized by high lethality rates, reaching 20% to 30%, even with adequate treatment (SOUZA, 2021). In addition, it can have high lethality when it affects patients from risk groups (elderly people, pregnant women, and individuals with deficient immunological conditions), with high rates: 70% in cases of meningitis, 50% of sepsis and greater than 80% in neonatal infections (DISSON et al., 2021; RODRIGUES et al., 2017).

In the USA, it is estimated that the incidence of listeriosis is between 0.1 and 10 cases per million inhabitants per year; however, this can be considerably different, according to the continents and countries of the world (WHO, 2024). In Brazil, it remains an underdiagnosed and underreported disease, which results in a lack of real data on the prevalence and epidemiology of the disease (OLIVEIRA et al., 2019; RODRIGUES, 2017).

The most common form of contamination by *L. monocytogenes* is via the oral route and the foods most involved in its transmission are industrialized ones, submitted to safe thermal treatments, and consumed without prior heating. Due to its high resistance to environmental conditions and the ability to form biofilms in processing equipment and machinery, which makes it difficult to prevent and control food contamination, its dissemination is often associated with the consumption of ready-to-eat products, that may allow the pathogen to proliferate during the production process and refrigerated storage (CAVALCANTI et al., 2022; PREUBEL et al., 2015).

Concerning tolerance limits in food, the United States adopted a zero-tolerance policy, not allowing the presence of the pathogen in any type of product (FAO & WHO, 2022). In the other hand, Brazilian Normative Instruction nº 161 established absence of *L. monocytogenes* in 25 g or mL in ready-to-eat foods and special formulas for parenteral nutrition, do not considering other types of products (BRASIL, 2022).

The identification of *L. monocytogenes* by conventional methods is based on bacteriological techniques, which involve enrichment in culture media and subsequent cultivation in selective media, followed by biochemical identification. These techniques require several days to complete; therefore, faster and more sensitive methods for the analysis of the pathogen in food have become available.

Although Brazil's epidemiological surveillance system for foodborne diseases is robust (BRASIL, 2021), there is a lack of data to illustrate the magnitude of the problem and reinforce the need for the application of control and prevention measures

for *L. monocytogenes* in food, since the country is an of the largest meat producers and exporters in the world. Due to the risks to public health and its importance in food, this work was carried out, to detect *L. monocytogenes* in animal products from different sources collected in slaughterhouses and dairies under the Federal Inspection Service (FIS) e equipment from meat sausage processing plants located in Brazil with Real-Time PCR technique and by the VIDAS LMO® Kit (BIOMÉRIEUX, Lyon, France).

## MATERIALS AND METHODS

### Sampling

Table 1 shows the 248 samples analyzed in the sources under study, including 95 of dairy products, 74 of chicken meat, 19 of frozen, 14 of beef cuts, 13 of mechanically deboned chicken meat, 13 equipment of Brazilian sausage processing plant, 12 of processed meat products and 08 of edible offal.

Five hundred grams were collected from samples of cheeses, whey powder, chicken meat, meat cuts, bovine giblets and meat products. The other dairy products (butter, condensed milk, dulce de leche paste) and frozen (ice cream, popsicles, and pies) were purchased in the original packaging, while the equipment was sampled with swabs moistened in 0.1% peptone water, using a 100 cm<sup>2</sup> template (10X10cm) for delimiting the area.

The samples were collected in March and April 2023, in 33 industries located in Goiás, Maranhão, Mato Grosso, Minas Gerais, Rondônia, Tocantins and the Distrito Federal. These were placed in isothermal boxes containing flaked ice and sent to and processed at the Food Research Center of the School of Veterinary and Animal Science at the Universidade Federal de Goiás (CPA/EVZ/UFG). The analyzes were carried out in the Food Microbiology (FML) and Molecular Biology (MBL) laboratories of this Center.

### Sample preparation

Initially, 25 g or 25 mL of each sample were homogenized in 225 mL of Modified *Listeria* Enrichment Broth (UVM) (Difco™) and the swabs were resuspended in 10 mL of UVM broth and incubated at 30 °C/24 h-26 h in a type BOD incubator. A 1 mL aliquot of the enriched cultures was transferred to 10 mL Fraser broth (FB-Fraser) (Difco™), followed by incubation as described. Then, 0.5 mL was transferred to the cap of the VIDAS LMO 2® equipment (BIOMÉRIEUX, Lyon, France), and the detection and confirmation of *L. monocytogenes* were performed according to the manufacturer.

Table 1 - Distribution of samples according to the sources under study for detection of *L. monocytogenes*.

Samples	Sample N°		
Dairy products	95		
Cheese	Mozzarella	48	
	Parmesan	7	
	Minas Frescal	7	
	Ricotta	4	
	Provolone	4	
	Minas Padrão	3	
	Rennet	2	
	Tropical	2	
	Kingdom	1	
	Piedmontese	1	
Cottage cheese	7		
Milk powder	3		
Butter	2		
Condensed milk	2		
Dulce de leche paste	1		
Whey powder	1		
Chicken meat	Cuts	65	
	Boneless chicken	8	
	Carcass	1	
Frozen	Ice cream	13	
	Popsicles	6	
Beef cuts	14		
-----Mechanically deboned chicken meat-----		13	
Equipment	Boning machines	4	
	Utensils	1	
	Sealer	1	
	Table	1	
	Shape	1	
	Block breaker	1	
	Press	1	
	Tank	1	
	Sausage peeler	1	
	Transport box	1	
	Processed meat products	Mixed mortadella	3
		Mixed sausage	3
		Chicken sausage	1
		Pork tripe	3
Fresh chicken sausage		2	
Edible offal	Chicken mortadella	1	
	Chicken offal	1	
	Bovine offal	7	

\*N°= Number of samples for each product.

*Listeria monocytogenes* testing – Real-Time PCR reaction and VIDAS LMO 2

Genomic DNA extraction was performed with the ‘High Pure PCR Template Preparation Kit’ (ROCHE®, Mannheim, Germany), with some modifications. A 1.5 mL volume of the UVM broth

culture was centrifuged (9,500 × g for 10 min.), the supernatant discarded, and the pellet resuspended in 450 µL of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). After adding 50 µL of lysozyme (10 mg/mL), the solution was incubated at 37 °C/2 h in a water bath for cell wall lysis. From this point onwards,

the kit manufacturer's recommendations were followed. DNA was eluted in 200 µL of elution buffer prewarmed to 70 °C and solution stored at -20 °C for later use.

To assess the integrity and concentration of the DNA, the DNA solution was electrophoresed on a 0.8% agarose gel in 0.5XTBE buffer (Tris-Borate-EDTA, pH 8.0), with a run at 80V/50 min.

λ-DNA/*Hind* III was used as molecular weight (MW) standard, gels stained with GelRed (Life Technologies™) and reading was performed on the Gel Doc XR system (BioRad, USA), with the aid of the Quantity One Software. amplification genomic DNA concentrations of 20ng were used for amplification (20 ng/5 µL). *L. monocytogenes* ATCC® 19114 strain was used as positive control and ultrapure water as a negative one.

List primers were used. Monocy-F-1 (5' CATGGCACCAACCAGCATC3'), List.monocy.-R-1 (5' CATCCGCGTGTTCCTTTTC3') (Genebank Accession Code: FF183456.1) and the probe List.Monocy.-S-1 (5' FAM CGCCTGCAAGTCTAAGACGC TAMRA3') (this research), which can be found. Located at positions 113-131, 178-158, and 134-154, respectively, of the *Hly* gene of *L. monocytogenes*, which encodes the production of listeriolysin O. The amplicon originated has 65 base pairs (bp). The synthesis of primers and probes was performed by Life Technologies™ (São Paulo, Brazil).

The Real-Time PCR reaction was performed using a volume of 25µL of the reaction mixture, contained 10 pmol of each primer, 6 pmol of probe, 20 ng of DNA, 12.5 µL of Taq Man Master Mix solution and 5.97 µL of ultra-pure water.

All of the amplification steps were carried out in a StepOnePlus Real-Time PCR System (Applied Biosystems California, USA.), with the following protocol: Initial denaturation (95 °C/5 min), followed by 40 heating cycles of denaturation (95 °C/15 sec), annealing (54 °C/1 min) and extension (60 °C/30 sec), and final extension (60 °C/30 sec). Samples that emitted curves exceeding the "threshold" line were considered positive.

## RESULTS AND DISCUSSION

Table 2 shows 7.3% (18/248) of *L. monocytogenes* in the sources studied by two methods. The highest percentage was verified in mechanically deboned chicken meat (MDCM), 84.6% (11/13) and processed meat products, 8.3% (01/12), followed by chicken meat and dairy products, with 4.0% (03/74) and 3.1% (03/95), respectively.

The positive results of dairy products were identified in fresh, dish, and mozzarella cheeses, not being present in cured (parmesan, provolone, standard Minas, rennet, Tropical, Kingdom and Piedmontese) and ricotta cheeses. Highlighting that all the samples analyzed were collected in industries under the Federal Inspection Service (SIF) of Brazil, which eliminate the chances of these cheeses having been made with unpasteurized milk. Therefore, it can be suggested that the presence of *L. monocytogenes* in such products could be attributed to post-processing contamination, environmental conditions, and/or their ability to form biofilms on equipment of food industry, a fact that harms the sanitation of surfaces that come into contact with food (EL-HAJJAJI et al., 2022), which makes it difficult to prevent and

Table 2 - Distribution of *L. monocytogenes* according to detection sources and analysis methods.

Source	----- <i>L. monocytogenes</i> -----		-----Real Time PCR-----		-----VIDAS LMO2-----	
	+	%	+	%	+	%
Dairy products	03/95	3.16	03/95	3.16	02/95	2.10
Chicken meat	03/74	4.05	03/74	4.05	02/74	2.70
Frozen	0/19	0.00	0/19	0.00	0/19	0.00
Beef cuts	0/14	0.00	0/14	0.00	0/14	0.00
MDCM	11/13	84.61	09/13	69.23	05/13	38.46
Equipment	0/13	0.00	0/13	0.00	0/13	0.00
Edible meat products	0/08	0.00	0/08	0.00	0/08	0.00
Processed meat products	01/12	8.33	01/12	8.33	01/12	8.33
Total	18/248	7.26	16/248	6.45	10/248	4.03

\* mechanically deboned chicken meat.

control product contamination. Another factor to be considered is failures during the pasteurization process since the three types of positive cheeses in our study are made with pasteurized milk. It is important to remember that, in the case of mozzarella, the dough is threaded during its preparation.

As for Minas fresh, characteristics of the cheese can favor the proliferation of the bacteria in the product, as it has high moisture content and can allow it to reach high numbers during storage and marketing, in addition to the fact that the products are consumed without prior heating (PINTO et al., 2020). These aspects, combined with possible storage failures in retail store and residence, give these products extreme importance in Public Health, since soft cheeses have already been associated with outbreaks of human listeriosis (CDC, 2018).

The presence of the bacteria was also reported in cheeses by APOLINÁRIO et al. (2014), who found 9.6% of positive samples for *L. monocytogenes* in products produced and marketed by dairy companies in Minas Gerais, results superior to those described here.

The high percentages in MDCM and the importance represented by such a finding should be highlighted, since it integrates the composition of practically all processed products, such as sausages,

mortadella, stakes, and nuggets, among others, although with quantity limits defined in the legislation. In this source, contamination can be due to raw material or in different stages of slaughter including bleeding, stirring, removal of internal organs, and immersion in water during the pre-chilling and chilling processes of the carcasses. positive samples of chicken cuts were obtained (Table 3), reinforcing the role of the carcasses themselves as a source of contamination of MDCM by *L. monocytogenes* already present during slaughter. Additionally, one of the parts of chicken carcasses with the most risk of contamination is the skeleton of broilers and layers used to produce MDCM, due to the high pH and water activity (aw) (KHALILI et al., 2019; AKRAMZADEH et al., 2020), characteristic of this source and which make it an extremely worrying substrate, since it is a highly fragmented raw material, which increases its availability and use by the pathogen.

In the present study, *L. monocytogenes* was identified in a fresh chicken sausage, which can probably be attributed to equipment contamination, since these may contain biofilms originated by colonization and failures in cleaning. It is noted that the presence of microorganisms in meat processing plants and refrigerated abattoirs is well documented, as well as the contamination by handlers (ALONSO

Table 3 - Positive samples for *L. monocytogenes*, according to the detection method.

Sample	Real Time PCR	VIDAS LMO2
1. mozzarella	+	+
2. mozzarella	+	-
3. fresh Minas cheese	+	+
4. chicken cut	+	+
5. chicken cut	+	-
6. chicken cut	+	+
7. mechanically deboned chicken meat	-	+
8. mechanically deboned chicken meat	+	+
9. mechanically deboned chicken meat	+	+
10. mechanically deboned chicken meat	+	-
11. mechanically deboned chicken meat	+	-
12. mechanically deboned chicken meat	+	-
13. mechanically deboned chicken meat	+	-
14. mechanically deboned chicken meat	+	-
15. mechanically deboned chicken meat	+	-
16. mechanically deboned chicken meat	+	+
17. mechanically deboned chicken meat	-	+
18. fresh chicken sausage	+	+

+ = positive sample.

- = negative sample.

et al., 2019; PALMA et al., 2016). In our research, all equipment samples analyzed were negative, which suggested that they are establishments with good hygiene and sanitation practices. It should be noted that the positive samples detected here, regardless of the type, came from others food industry.

Contrary to what was verified in the present research, in which the analyzed samples of beef cuts were negative, PALMA et al. (2016) detected *L. monocytogenes* in 8.9% (11/125) bovine meat cuts analyzed in the Distrito Federal, Brazil. The authors also used Real-Time PCR technique.

SERENO et al. (2019) in a study of 824 environmental, carcass and food samples obtained at different stages of the production chain and the slaughterhouse, obtained 87 positive samples for *L. monocytogenes* using the PCR technique. The bacterium was detected in environmental samples from the slaughter stages (drains, 2/20) and the cutting room (conveyor belts in the final packaging stage - 11/20), obtaining results in line with the present study.

Table 1 also shows the distribution of *L. monocytogenes* in the different sources, according to the adopted methodology. When using Real-Time PCR, 6.4% (16/259) samples were positive, being 3.1% (03/95) of dairy products, 4.0% (3/74) of chicken meat, 69.2% (09/13) of MDCM, and 8.3% (01/12) of processed meat product. With VIDAS LMO 2, 4.0% (10/248) were positive in the total analyzed, being 2.1% (02/95) of dairy products, 2.7% (02/74) of chicken meat, 38.4% (05/13) of MDCM, and 8.3% (01/12) of processed meat products. The absence of bacteria in frozen, beef cuts, and edible offal and equipment was observed using both methodologies (Table 2).

Considering the techniques used (Table 2), a higher frequency of occurrence was observed when using Real-Time PCR, which was also described by TANG (2011). In the present study, the results obtained with this methodology were superior to those verified with VIDAS LMO 2 in all sources in which the bacterium was present, except in processed meat products, in which the positive sample was detected by both techniques.

Conversely, LEDLOD et al. (2020) in a study to detect *Listeria* spp. and *L. monocytogenes* in 100 meat samples, obtained a positive result in 6% of the samples. When comparing the sensitivity between the techniques, 100% accuracy was demonstrated against other standard detection methods, VIDAS and PCR, differing from the present study.

Considering positive samples obtained with the two methodologies (Table 3), it should

be noted that seven were identified by the two techniques, eight only by Real-Time PCR and two only by VIDAS LMO 2, requiring the association of the two methods to obtain the total of positive.

Of the positive by Real-Time PCR, one was from mozzarella cheese, one from commercial chicken cut, and six chicken MDCM; those positive exclusively by VIDAS LMO 2, two were from MDCM (Table 3).

In Brazil, many authors reveal the common presence of *L. monocytogenes* in raw milk, and the health risk of these raw materials should be highlighted given the frequency of their use in the production of cheeses from non-pasteurized milk, a common practice in the informal food trade in the country and in cheeses with the Arte Seal. In raw milk samples from an expansion tank in the state of Paraná, Brazil, a high frequency of *L. monocytogenes* was identified from bacteriological analysis, since in seven of the 10 samples it was possible to identify the agent (CAVALLI et al., 2016). Similarly, in a study carried out by GONÇALVES (2017) with soft cheeses containing around 70% raw sheep's milk, the presence of *Listeria* spp. was detected in 30% (09/30) of samples and, of these, 13.3% (4/30) had *L. monocytogenes*, three with counts of more than 100 cfu/g. It should be noted that, in our study, *L. monocytogenes* was identified in three samples of cheeses obtained from pasteurized milk (Table 3).

DING et al. (2017) in a study of 46 environmental and raw milk samples from farms in China, using the Real-Time PCR technique, obtained four (8.7%) of the samples positive for *L. monocytogenes*, concluding that the technique has a good detection limit, whether prior enrichment of the samples is used. Similar to the one obtained here, the authors also observed the superiority of the latter technique. For them, this proved to be efficient, capable of amplifying and detecting a reduced number of bacterial cells in the source under study.

Corroborating our results, RÍOS-CASTILLO et al. (2020) compared the conventional microbiological method with VIDAS and Real-Time PCR for the detection of *L. monocytogenes* and concluded that the latter proved to be more sensitive for detection than the conventional method.

In a study to analyze the methods used to detect *L. monocytogenes*, SOUZA (2017) describes that the most recurrent methods for identification were based on bacteriological and PCR techniques, with 32.6% each, followed by commercial kits: 21.5% API *Listeria*<sup>®</sup> (Biomérieux) and 7.4% VIDAS<sup>®</sup>. Other techniques represented 5.9% of the studies included.

There is greater sensitivity in PCR-based tests when compared to results obtained in culture methods, and greater specificity in the PCR technique, in line with what was also obtained in the present research and already demonstrated by LEDLOD et al. (2020) who stressed that the developed PCR protocol showed high specificity for *L. monocytogenes* in meat foods. In our research, of the 18 positive samples detected, 16 were obtained using Real-Time PCR, while with VIDAS LMO 2, 10/18 (Table 2).

The greater efficiency of the PCR technique for detecting this pathogen in food, verified by many authors, can be attributed to several factors, the sensitivity and specificity of the method being of interest. Other relevant aspects to consider are the certainty of the viability of the microorganisms, detected after enrichment and/or pre-enrichment of samples in specific culture media (LIU et al., 2019), as well as greater speed in obtaining results, a fact of great relevance in the routine of food industries.

The ability to detect the pathogens, even when present in low numbers, combined with correct identification and rapid results obtained has made the methodology of great value for the food industries, due to the characteristics of food matrices and the particularities of many microorganisms of interest in food and in Public Health, many of which can grow slowly e/or fastidiously, are viable but not cultivable, are difficult to identify or have very long generation times (SANTOS et al., 2024). As it is a psychrotrophic microorganism, research and identification of *L. monocytogenes* using a conventional bacteriological method can take days to weeks (ISO, 2017).

The greater number of positive samples obtained when using Real-Time PCR may be due to its greater detection threshold, which this technique being able to detect a small number of cells.

In terms of Public Health and the risk of listeriosis due to the consumption of foods contaminated by the pathogen has already been verified, the risk in cheeses is high as it is often consumed without additional heat treatment. The risks are also high when it comes to the meat that is consumed rare, especially sausage and chicken, products traditionally consumed in barbecues in Brazil, which reinforces the problem of risks to the health of the population.

The main food sources of *L. monocytogenes* are those of animal origin, meat, and dairy products, as highlighted by numerous authors (POUILLOT et al., 2020), and in Brazil, meat products were identified by VALLIM et al. (2015), after analyzing the occurrence of *Listeria* spp. in foods purchased in 12 Brazilian states in the period between 1990 and 2012, in which

they concluded that 81.7% of the isolates of the species were of meat origin, with 24.3% detected in cooked, cured or smoked meats. Similarly, in the present study the species was detected in both dairy and meat products, highlighting its presence in mechanically deboned chicken meat, a raw material used on a large scale by industries, and which is part of the composition of a large number of processed meat products.

In research on the distribution and persistence of the bacteria in Canadian pig abattoirs, it was possible to identify a high prevalence of *L. monocytogenes* in the cutting and deboning operation area, inferring the risk of meat contamination by persistent strains in the operation area (CHERIFI et al., 2020), highlighting the importance of identifying the presence of in the processing environment and mitigating the potential for contamination of food (MAGDOVITZ et al., 2020).

In Brazilian Normative Instruction nº 161, there are microbiological standards established for *L. monocytogenes* only for ready-to-eat foods and for ready-to-eat foods intended for infants or for special purposes. Additionally, products that fit into specific situations are not required from the regular research of the pathogen, especially those with a shelf life of fewer than five days; foods with a pH less than or equal to 4.4; foods with water activity less than or equal to 0.92; foods with a combination of pH less than or equal to 5.0 and water activity less than or equal to 0.94, among others. It is adopted is the absence of *L. monocytogenes* in 25 g (BRASIL, 2022).

However, the Ministry of Agriculture Livestock and Food Supply (MAPA), through Normative Instruction (IN) 09, of April 8, 2009 (BRASIL, 2009), instituted the Procedures for the Control of *L. monocytogenes* in ready-to-eat products of animal origin. This IN aims to ensure the safety of these products in relation to this pathogen and applies to establishments that manufacture ready-to-eat products of animal origin that have the following physicochemical characteristics: pH>4.4 (greater than four point four) or Water Activity>0.92 (greater than zero point ninety-two) or sodium chloride concentration<10% (less than ten percent). 4 (greater than four point four) or Water Activity>0.92 (greater than zero point ninety-two) or sodium chloride concentration <10% (less than ten percent), respecting the characteristics of their production processes.

MAPA also determine that cooked and smoked hams, bologna, cooked sausages, cooked and smoked sirloin and palette, soft and semi soft cheese, smoked fish, surimi, cooked and frozen bivalve mollusk and shrimp are officially monitored by a

national sampling plan in industries under federal inspection (BRASIL, 2013). Besides that, the Federal Inspection Service (SIF) monitors the industries to check the use of appropriate tools aiming to ensure the safety of meat, dairy and fishery products, such as GMP, SSOP and HACCP.

In the present research, positive samples were detected, by the two techniques, in mozzarella cheeses, fresh Minas cheese fresh, chicken meat cuts, fresh chicken sausage, and mechanically deboned chicken meat (Table 1, Table 3). These results are quite worrying because it is a highly lethal pathogen, which; although, not thermally resistant, may survive and cause listeriosis due to the consumption of thermally under-processed or even raw foods, reinforcing the need for attention by the organs inspection and regulation, since in Brazil there is no legislation for meat and other products except for the afore mentioned (BRASIL, 2022).

## CONCLUSION

The study highlighted the risk of the presence of *L. monocytogenes* in foods of the animal origin and the importance of monitoring it to reduce the risks of its transmission through these foods. VIDAS and Real-Time PCR technique proved to be satisfactory tests for detecting these bacteria.

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## DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

## AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically reviewed the manuscript and approved the final version.

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