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Occurrence of *Listeria* spp. and *Listeria monocytogenes* in half-carcasses, meat cuts, equipment, and the environment of bovine slaughterhouses in Brazil

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ABSTRACT

Human listeriosis is a severe food-borne illness, with fatality rates ranging from 20 to 30 %. In Brazil, despite being an underdiagnosed and underreported disease, the presence of the microorganism in food has been the subject of important studies. However, its occurrence in slaughterhouse environments has received little attention in recent years. Therefore, the aim of this study was to determine the occurrence of *Listeria* spp. and *L. monocytogenes* in samples of environments (boning rooms, cold rooms, and slaughter rooms), equipment, half-carcasses, and retail cuts. Samples were collected from 25 slaughterhouse units belonging to five industries under the Federal Inspection Service (SIF) licensed for international trade and located in Brazilian states (São Paulo, Mato Grosso do Sul, Goiás, Mato Grosso, Bahia, Pará, and Minas Gerais), using Polymerase Chain Reaction (PCR). Primers pairs U1/LI1 for *Listeria* spp., and LM1/LM2 and LL5/LL6 (Invitrogen©) for L. *monocytogenes* were used for amplification. *Listeria* spp. was detected in 19.7 % (71/360) of the total samples analyzed, with detection rates of 23 % in deboning rooms, cold rooms, and half-carcasses, 15 % in meat cuts, and 10 % in slaughter rooms. In the total analyzed, *L. monocytogenes* was detected in 14.7 % (53/360) of the samples, with rates of 20 % in cold rooms, 15.8 % in boning rooms, 13.3 % in half-carcasses and meat cuts, and 10 % in slaughter rooms. The LM1/LM2 primer pair proved more efficient than LL5/LL6. The results of this study highlight the need for urgent measures to control the pathogen in cattle slaughterhouses in Brazil.

1. Introduction

Human listeriosis is a rare disease caused by *Listeria monocytogenes* and also by *L. ivanovii*, and can occur in invasive and non-invasive forms. The invasive form can cause meningitis, septicemia, abortion, meningoencephalitis, and endocarditis, usually affecting elderly people, pregnant women, and individuals with compromised immune systems (Kalani et al., 2018; Radoshevich and Cossart, 2018), while the non-invasive form causes mild, flu-like symptoms (Henkel et al., 2015).

Listeriosis is characterized by high lethality rates (20 % to 30 %) (Souza et al., 2021). It can be more severe in individuals from risk groups, with high fatality rates: 70 % in meningitis cases, 50 % in sepsis, and over 80 % in neonatal infections (Disson et al., 2021; Rodrigues et al., 2017).

The genus includes *L. aquatic*, *L. booriae*, *L. cornellensis*, *L. denitrificans*, *L. fleischmannii*, *L. floridensis*, *L. grandensis*, *L. grayi*, *L. innocua*, *L. marthii*, *L. murrayi*, *L. newyorkensis*, *L. riparia*, *L. rocourtiae*, *L. seeligeri*, *L. weihenstephanensis*, and *L. welshimeri*, but only *L. monocytogenes* and *L. ivanovii* are pathogenic (Parte et al., 2020; Quereda et al., 2021).

Several factors, such as prevalence, resistance, and severity due to the high mortality rate in immunocompromised individuals, justify the evaluation of *Listeria* spp. and *L. monocytogenes* in food matrices, especially meat products (Demaître et al., 2020; Odu and Okonko, 2017;

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Townsend et al., 2021).

Contamination of meat by this microorganism can occur at various stages of the food chain, but it is most common during animal slaughter in slaughterhouses, as animals are considered a significant source of microbial contamination (Santana et al., 2025). Contamination may result from inadequate handling practices and failures in sanitization processes. This is attributed to its ability to survive under adverse conditions, grow at refrigeration temperatures, resist freezing, and form biofilms in the food industry, which contributes to resistance to various sanitizers (Davanzo et al., 2021; Demaître et al., 2020; Gebremedhin et al., 2021).

Due to the risk posed by the presence of *L. monocytogenes*, regulatory bodies, along with researchers and the food industry in several countries, have increased their focus on contamination by this microorganism in processing plants and in foods of both animal and plant origin (Farber et al., 2021; Simonetti et al., 2021; Tadielo et al., 2023; Tan et al., 2019).

In Brazil, microbiological guidelines for food are provided for in Normative Instruction (IN) No. 161/2022 (Brasil, 2022a) and Resolution RDC 724/2022 (Brasil, 2022b), which set limits for *L. monocytogenes* in ready-to-eat foods. The maximum limit is 10^2 CFU/g or mL in ready-to-eat foods, and absence is required in those intended for infants and in special formulas for parenteral nutrition.

Additionally, products falling under specific categories are exempt from routine testing for the pathogen, especially those with a shelf life of fewer than five days; foods with pH $\leq$4.4; foods with water activity $\leq$0.92; foods combining pH $\leq$5.0 and water activity $\leq$0.94; and products that undergo heat treatment or an equivalent process that eliminates *L. monocytogenes* and for which recontamination after processing is not possible, such as heat-treated products in their final packaging (Brasil, 2022a).

In Brazil, physicians and other healthcare professionals, as well as those responsible for public and private health institutions, are legally required to report outbreaks of foodborne diseases (FBD) to the government (Brasil, 2010).

The implementation of interventions and sanitary measures for the prevention and control of FBD outbreaks must follow specific legislation established by the Ministry of Health, the National Health Surveillance Agency, and the Ministry of Agriculture and Livestock. In addition, there are specific regulations for FBD surveillance, which guide notification and control procedures.

The Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA), through Normative Instruction No. 09/2009 (Brasil, 2009), established the Procedures for the Control of *L. monocytogenes* in ready-to-eat products of animal origin. These procedures aim to ensure the safety of such products regarding this pathogen and apply to establishments that manufacture ready-to-eat products of animal origin with the following physicochemical characteristics: pH >4.4, water activity >0.92, or sodium chloride concentration < 10 %, respecting the characteristics of each production process.

MAPA also determines that cooked and smoked hams, bologna, cooked sausages, cooked and smoked sirloin and shoulder, soft and semi-soft cheeses, smoked fish, surimi, cooked and frozen bivalve mollusks, and shrimp are officially monitored through a national sampling plan in industries under federal inspection (Brasil, 2013). In addition, the Federal Inspection Service (SIF) monitors these industries to ensure the use of appropriate tools to guarantee the safety of meat, dairy, and fishery products, such as Good Manufacturing Practices (GMP), Sanitation Standard Operating Procedures (SSOP), and Hazard Analysis and Critical Control Point (HACCP).

Although Brazil's epidemiological surveillance system for foodborne diseases is robust (Brasil, 2021), there is a lack of data to illustrate and reinforce the need for control and prevention measures, particularly concerning *L. monocytogenes*, as there are no officially reported cases of human listeriosis in the country. However, as demonstrated by Iglesias et al. (2022), who evaluated the serogroups of *L. monocytogenes* isolates obtained from 200 bovine carcass samples from slaughterhouses in the Rio Grande do Sul region, Brazil, it was observed that *L. monocytogenes* strains persist over time in beef processing facilities. Among the isolates, serogroups IIb and IVb were present, indicating a high invasive capacity in HCT cells and suggesting their potential to cause invasive human listeriosis.

Studies conducted in Brazil, such as the one by Santana et al. (2025), analyzed 248 samples of dairy products, chicken meat, edible ice cream, beef cuts, mechanically separated chicken meat, fresh offal, processed meat products, and equipment in Brazilian food industries. The study detected *L. monocytogenes* in 6.4 % (16/248) of the samples using Real-Time Polymerase Chain Reaction (PCR), demonstrating the presence of this microorganism in both ready-to-eat foods and raw meat cuts.

Although *L. monocytogenes* is widely studied, there are few reports on its monitoring in cattle slaughterhouses qualified for international trade in Brazil (Camargo et al., 2015; Iglesias et al., 2017; Loiko et al., 2016; Voloski et al., 2016). Therefore, this study aims to detect *Listeria* spp. and *L. monocytogenes* using the PCR technique, and to determine contamination levels and their distribution in the environments and equipment of cattle slaughterhouses, as well as on the surfaces of half-carcasses and meat cuts from these establishments.

2. Materials and methods

2.1. Sampling

The sampling consisted of convenience samples of a non-probabilistic nature. Environmental and equipment samples were selected based on their identification as common sources of *Listeria* spp. contamination in cattle slaughterhouses. These samples were collected monthly (January to July 2022) as part of a monitoring program in Brazilian slaughterhouses. Collections were carried out by different units during slaughter shifts, randomly and according to each company's schedule, and samples were forwarded to the Molecular Biology Laboratory of the Food Research Center at the School of Veterinary Medicine, Federal University of Goiás.

Samples were sent by industrial units from five slaughterhouses located in the states of São Paulo, Mato Grosso do Sul, Goiás, Mato Grosso, Bahia, Pará, and Minas Gerais (Fig. 1). It is important to emphasize that none of these establishments performed any decontamination procedures on carcasses or meat cuts. (See Fig. 2.)

The environmental and equipment samples included 120 from boning rooms (conveyor belts and rollers, evaporators, and drains), 60 from cold rooms (drains and evaporators), and 60 from slaughter rooms (drains and rollers). In addition, 60 samples were collected from the surface of half-carcasses (hot and chilled) and 60 from meat cuts (vacuum-packed and unpackaged). Thirty samples were analyzed from each site or source mentioned (Table 1).

Samples from the environment, equipment, half-carcass surfaces, and unpackaged meat cuts were collected using swabs moistened with 0.85 % saline solution (pH = 7.0), swabbing areas ranging from 10 to 100 cm^2, depending on the surface available.

In the case of half-carcasses, samples were collected after carcass division. For refrigerated cuts, sampling occurred after complete cooling, just before they left the cold chambers. The sampled areas included the sirloin, shoulder, and rump. Unpackaged meat cuts were obtained after deboning, while vacuum-packed cuts were sampled after packaging.

Once identified, the samples were placed in isothermal boxes containing flaked ice and sent to the Laboratory of Molecular Biology at the Food Research Center of the School of Veterinary Medicine, Federal University of Goiás (CPA/EV/UFG). Vacuum-packed meat cuts were also transported in isothermal boxes, ensuring the physical integrity of the packaging and maintaining low temperatures until arrival.

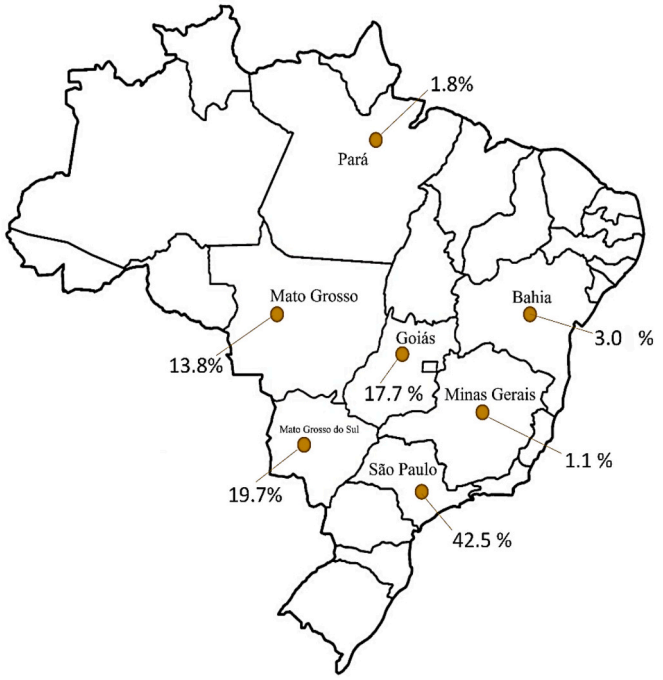

Fig. 1. Map of Brazil showing the locations where the slaughterhouse/packing plant samples were collected along with the percentage of samples received from each region.

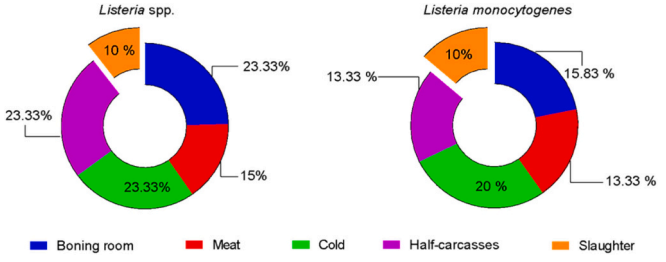

Fig. 2. Distribution of *Listeria* spp. and *L. monocytogenes* across different sources.

Table 1
Source and number of samples evaluated on equipment and carcass surfaces in beef slaughterhouses.*

Site	Collection point	N
Equipment and environments ($n = 240$)		
Boning room ($n = 120$)	Conveyor	30
	Roulette of conveyor	30
	Evaporators	30
	Drains	30
Chilling room ($n = 60$)	Evaporators	30
	Drains	30
Slaughter room (n = 60)	Roulette of conveyor	30
	Drains	30
Half carcasses and cuts (n = 120)	Hot half-carcass surface	30
	Chilled half-carcass surface	30
	Vacuum-packed cut	30
	Unpackaged cuts	30

* N = number of samples collected per sampling.

2.2. Conventional PCR technique

2.2.1. Sample preparation and isolation of total DNA

Aliquots of 2 mL of the sample, resuspended in 0.85 % saline solution, were pre-enriched in 20 mL of tryptic soy broth (Casoy; Himedia, India) at 28 °C for 18 h in a Biological Oxygen Demand (BOD) incubator. After this period, 2 mL of the culture was centrifuged at 10,000 rpm for 10 min, the supernatant was discarded, and 500 µL of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and 50 µL of lysozyme (10 mg/mL) were added to the pellet. The samples were homogenized and incubated in a water bath at 37 °C for 24 h. The same procedures were applied to meat cuts, using 2 mL of exudate for cultivation.

Genomic DNA was isolated as described by Lawrence and Gilmour (1994), and the resulting pellet was hydrated with 100 µL of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and stored at −20 °C. DNA concentration was estimated by electrophoresis on a 0.8 % agarose gel in 0.5× TBE (Tris-Borate-EDTA) buffer, using 2 µL of DNA solution from each sample, run at 80 V for 30 min. Photographic documentation was performed using the Gel Doc XR system (Bio-Rad) with Quantity One software (Bio-Rad, California, USA).

2.3. PCR amplification

For the analysis, 100 ng of DNA was used for amplification. Positive and negative controls for the PCR reactions were *L. monocytogenes* ATCC 19111 (American Type Culture Collection – ATCC, Microbiologics, Plastlabor) and *L. innocua* ATCC 33090 (Microbiologics, Plastlabor), used in reactions with the respective species-specific primers. Both ATCC strains were used as positive controls for genus-level amplifications, while ultrapure water served as the negative control.

Primer pairs U1/LI1 for *Listeria* spp., and LM1/LM2 (Lawrence and Gilmour, 1994) and LL5/LL6 (Golsteyn Thomas et al., 1991) for *L. monocytogenes*, were used. The oligonucleotides were synthesized by Invitrogen® (Thermo Fisher, São Paulo, Brazil). Primers U1 (5′ CAGCMGCCGCGGTAATWC 3′) and LI1 (5′ CTCCATAAAGGTGACCCT 3′) are located at positions 519–536 and 1457–1440, respectively, of the 16S subunit of *Listeria* spp. rRNA, yielding a 938-bp amplicon. Primers LM1 (5′ CCTAAGACGCCAATCGAA 3′) and LM2 (5′ AAGCGCTTGCAACTGCTC 3′) produce a 702-bp amplicon, while LL5 (5′ AACCTATCCAGGTGCTC 3′) and LL6 (5′ CTGTAAGCCATTTCGTC 3′), located at positions 372–389 and 622–639, respectively, yield a 268-bp amplicon. Amplification conditions followed the recommendations of Golsteyn Thomas et al. (1991) and Lawrence and Gilmour (1994).

The amplified products were subjected to 2 % agarose gel electrophoresis in 0.5× TBE buffer at 130 V for 40 min, using a 100 bp DNA Ladder (Invitrogen®) as the molecular weight (MW) standard. Gels were stained with GelRed, and photographic documentation was performed using the Gel Doc XR system (Bio-Rad, California, USA).

3. Results

3.1. Distribution of Listeria spp. and Listeria monocytogenes in the different sources

Table 2 shows the distribution of *Listeria* spp. and *L. monocytogenes* across the analyzed sources and sites, as determined by the PCR technique following non-selective enrichment in TSB broth at 28 °C for 18 h. Of the total samples analyzed, 19.7 % (71/360) were positive for *Listeria* spp., and 14.7 % (53/360) for *L. monocytogenes*.

Regarding *Listeria* spp. detection, 23.3 % (28/120) of the samples were positive in deboning rooms, 15 % (09/60) in meat cuts, 23.3 % (14/60) in cold rooms and half-carcasses, and 10 % (06/60) in the slaughter room. For *L. monocytogenes*, detection rates were 15.8 % (19/120) in deboning rooms, 13.3 % (08/60) in meat cuts, 20 % (12/60) in cold rooms, 13.3 % (08/60) in half-carcasses, and 10 % (06/60) in the slaughter room.

Among the 71 samples positive for *Listeria* spp., 18 were positive only at the genus level (data not shown). These included nine from boning rooms (two from rollers, three from drains, two from conveyors, and two from evaporators), one from vacuum-packed meat cuts, two from cold

Table 2
Distribution of *Listeria* spp. and *L. monocytogenes* in samples from Brazilian slaughterhouses, according to the sources of detection, using the PCR technique.

SOURCE	*Listeria* spp.		*L. monocytogenes*	
	(+)		(+)	
	N	%	N	%
BONING ROOMS	**28/120**	**23.3**	**19/120**	**15.8**
DRAINS	08/30	26.7	05/30	16.7
EVAPORATORS	06/30	20.0	04/30	13.3
STEELERS	08/30	26.7	06/30	20.0
CONVEYOR ROLLERS	06/30	20.0	04/30	13.3
MEAT CUTS	**09/60**	**15.0**	**08/60**	**13.3**
WITHOUT PACKAGING	05/30	16.7	04/30	13.3
VACUUM-PACKED	04/30	13.3	04/30	13.3
COLD-CHAMBERS	**14/60**	**23.3**	**12/60**	**20.0**
DRAINS	07/30	23.3	07/30	23.3
EVAPORATORS	07/30	23.3	05/30	16.7
HALF-CARCASSES	**14/60**	**23.3**	**08/60**	**13.3**
HOT	10/30	33.3	05/30	16.7
CHILLED	04/30	13.3	03/30	10.0
SLAUGHTER ROOMS	**06/60**	**10.0**	**06/60**	**10.0**
DRAINS	02/30	6.7	02/30	6.7
SKIN ROLLERS	04/30	13.3	04/30	13.3
TOTAL	**71/360**	**19.7**	**53/360**	**14.7**

*N = number of samples; (+) positive sample.

room evaporators, and six from half-carcasses—five from hot and one from chilled carcasses.

3.2. Efficiency of primer pairs for detecting L. monocytogenes in the sources under study

Table 3 presents the results obtained using two primer pairs for detecting *L. monocytogenes* in positive samples from different sources. Of the 53 positive samples identified, 92.4 % (49/53) were detected using the LM1/LM2 primer pair and 37.0 % (20/53) using LL5/LL6. By sampling source, LM1/LM2 detected 94.7 % (18/19) of positives in boning rooms, 87.5 % (07/08) in meat cuts, 91.7 % (11/12) in cold rooms, 87.5 % (07/08) in half-carcasses, and 100 % (06/06) in the slaughter room.

Table 3
Distribution of samples positive for *L. monocytogenes* in the different sources. According to the primer pairs used in PCR technique.[1]

SOURCE L. *monocytogenes*				
	LM1/LM2		LL5/LL6	
	(+)		(+)	
	N°	%	N°	%
BONING ROOMS	**18/19**	**94.7**	**06/19**	**31.6**
DRAINS	05/05[2]	100.0	01/05	20.0
EVAPORATORS	03/04	75.0	03/04	75.0
STEELERS	06/06	100.0	01/06	16.7
CONVEYOR ROLLERS	04/04	100.0	01/05	20.0
MEAT CUTS	**07/08**	**87.5**	**03/08**	**37.50**
WITHOUT PACKAGING	04/04	100.0	0/04	0.0
VACUUM PACKED	03/04	75.0	03/04	75.0
COLD CHAMBERS	**11/12**	**91.7**	**08/12**	**66.7**
DRAINS	06/07	85.7	05/07	71.4
EVAPORATORS	05/05	100.0	03/05	60.0
HALF-CARCASSES	**07/08**	**87.5**	**02/08**	**25.0**
HOT	04/05	80.0	01/05	20.0
CHILLED	03/03	100.0	01/03	33.3
SLAUGHTER ROOMS	**06/06**	**100.0**	**01/06**	**16.7**
DRAINS	02/02	100.0	0/02	0.0
SKIN ROLLERS	04/04	100.0	01/04	25.0
TOTAL	**49/53**	**92.5**	**20/53**	**37.0**

[1] Ratio between the number of positive samples detected with the primer pair and the total number of positives identified in the study at each source; [2]Ratio between the number of positive samples detected with the primer pair and the total number of positives identified in the study at each site sampled.

The detailed distribution of *Listeria* spp. and *L. monocytogenes* positive samples by source and primer pair is shown in Box 1.

Of the 49 samples positive with LM1/LM2, 33 were exclusively detected by this pair (data not shown). These included three from belt rollers, four from boning room drains, five from boning belts, one from an evaporator, four from unpackaged cuts, one from a vacuum-packed cut, two from cold room drains, two from cold room evaporators, four from hot and two from chilled half-carcasses, two from slaughter room drains, and three from skin rollers.

For the LL5/LL6 pair, 31.6 % (06/19) of positives were in boning rooms, 37.5 % (03/08) in meat cuts, 66.7 % (08/12) in cold rooms, 25 % (02/08) in half-carcasses, and 16.7 % (01/06) in the slaughter room. Table 3 also details the specific sampling points for each source.

Of the 20 positive samples identified by LL5/LL6, four were detected exclusively with this primer pair: one from a boning room evaporator, one from a vacuum-packed meat cut, one from a cold room drain, and one from a hot half-carcass (Box 1). Of the 53 samples positive for *L. monocytogenes* in this study, only 16 were detected simultaneously by both primer pairs: five from boning rooms, two from meat cuts, seven from cold rooms, one from a half-carcass, and one from the slaughter room.

4. Discussion

4.1. Distribution of Listeria spp. and L. monocytogenes in the different sources

The results demonstrate the occurrence and distribution of *Listeria* spp. and *L. monocytogenes* across all analyzed sources. The high detection percentages are noteworthy. The highest rates of *Listeria* spp. were found in deboning rooms, cold rooms, and half-carcasses (23.3 %), while *L. monocytogenes* was most frequently detected in cold rooms (20 %) and deboning rooms (15.8 %) (Table 2). These environments are rich in organic matter, and sanitization in boning rooms typically occurs only at the end of the day or shift, unlike the routine followed in slaughter rooms. Additionally, cold rooms are generally cleaned and sanitized only once a month. These factors likely explain the observed differences in detection rates across the three sources and the high prevalence found in boning and cold rooms.

The presence of *Listeria* spp. and *L. monocytogenes* in high percentages in all sources and sampling sites studied (Table 2) reveals their wide distribution and persistence in the slaughterhouses included in this study. The persistence of the genus *Listeria* and *L. monocytogenes* in slaughterhouses and food processing plants has generally been attributed by several researchers to the ability of these microorganisms to form biofilms in such environments (Chen et al., 2022, 2023; Tuytschaever et al., 2025; Yang et al., 2016). Additionally, other studies (Byun et al., 2022; Ramires et al., 2021) have emphasized failures in hygiene procedures adopted by the industry.

These aspects may help explain the results described here (Table 2) and suggest that the characteristics and adaptive abilities developed by these microorganisms are, at least in part, responsible for the levels of occurrence observed in this study.

It is well established that the washing and sanitizing procedures employed can contribute to biofilm formation—a common survival strategy used by the genus *Listeria* (Chen et al., 2022) in these environments and on installed equipment, such as conveyor rollers, belts, evaporators, and others (Byun et al., 2022; Ramires et al., 2021). These biofilms support bacterial persistence in the environment and on said equipment by impeding the removal of organic matter and reducing the efficacy of sanitizing agents (Chen et al., 2023).

The industrial environment can serve as an important source of *L. monocytogenes* contamination (Barancelli et al., 2011), and biofilms are recognized as significant reservoirs of pathogenic microorganisms in food production environments (Tuytschaever et al., 2025; Yang et al., 2016).

Box 1
Distribution of *Listeria* spp. and *L. monocytogenes* according to isolation sources and primer pairs used.

SAMPLES		*Listeria* spp.	*L. monocytogenes*	
		(U1/LI1)	LM1/LM2	LL5/LL6
BONING ROOMS	**CONVEYOR ROLLERS**			
	01 RED	+	−	−
	02 RED	+	+	+
	06 RED	+	+	−
	07 RED	+	+	−
	17 RED	+	−	−
	30 RED	+	+	−
	DRAINS			
	34 RD	+	+	−
	35 RD	+	−	−
	36 RD	+	+	−
	39 RD	+	−	−
	48 RD	+	+	−
	51 RD	+	−	−
	52 RD	+	+	−
	55 RD	+	+	+
	STEELERS			
	61 ED	+	+	−
	69 ED	+	−	−
	70 ED	+	+	−
	75ED	+	−	−
	83ED	+	+	−
	84ED	+	+	+
	85ED	+	+	−
	87ED	+	+	−
UNPACKAGED AND VACUUM-PACKED MEAT CUTS	**EVAPORATORS**			
	93EVD	+	+	+
	95EVD	+	+	−
	98EVD	+	−	−
	101EVD	+	−	+
	105EVD	+	+	+
	113EVD	+	−	−
	UNPACKAGED MEAT CUTS			
	4CNE	+	−	−
	15CNE	+	+	−
	16CNE	+	+	−
	20CNE	+	+	−
	25CNE	+	+	−
	VACUUM-PACKED MEAT CUTS			
	44CEV	+	+	+
	50CEV	+	−	+
	51CEV	+	+	−
	54CEV	+	+	+
COLD-CHAMBERS	**DRAINS**			
	2 RCF	+	+	+
	10 RCF	+	+	+
	14 RCF	+	+	−
	15 RCF	+	+	+
	16 RCF	+	+	+
	18 RCF	+	+	−
	21 RCF	+	−	+
	EVAPORATORS			
	36ECF	+	+	−
	37ECF	+	+	+
	38ECF	+	−	−
	42ECF	+	+	+
	43ECF	+	+	+
	55ECF	+	−	−
	57ECF	+	+	−
HOT AND CHILLED HALF-CARCASSES	**HOT HALF-CARCASS**			
	5 MCQ	+	−	−
	6 MCQ	+	−	−
	8 MCQ	+	−	−
	9 MCQ	+	−	−

(*continued on next page*)

(*continued*)

SAMPLES		*Listeria* spp.	*L. monocytogenes*	
		(U1/LI1)	LM1/LM2	LL5/LL6
	13MCQ	+	−	+
	18MCQ	+	+	−
	21MCQ	+	+	−
	22MCQ	+	−	−
	24MCQ	+	+	−
	30MCQ	+	+	−
	CHILLED HALF-CARCASS			
	31MCR	+	+	+
	35MCR	+	+	−
	48MCR	+	−	−
	52MCR	+	+	−
	DRAINS			
	12RA	+	+	−
	28RA	+	+	−
	SKIN ROLLERS			
	34RTA	+	+	+
	35RTA	+	+	−
	52RTA	+	+	−
SLAUGHTER ROOMS	59RTA	+	+	−

*(+) positive and (−) negative sample.

We suggest that these factors may account for the high percentages of the microorganism found in the studied environments and sampled equipment, particularly the drains, which exhibited higher frequencies of both *Listeria* spp. and *L. monocytogenes* compared to nearly all other sampling sites. It is also important to note that the cleaning and sanitizing practices in these areas may have contributed to the accumulation of organic matter in the drains, creating a highly favorable niche for psychrotrophic and facultative bacteria. These conditions align perfectly with the requirements and survival traits of *Listeria* spp. and *L. monocytogenes*, supporting their colonization and persistence in drains and the production environment (Chen et al., 2022).

The detection of *L. monocytogenes* in the industrial environment, as observed in this study (Table 2), is particularly concerning given the high percentages found on half-carcasses and meat cuts. These findings highlight the need for strict hygiene and sanitization protocols, as well as the importance of implementing and maintaining effective control measures.

The results are in agreement with the psychrotrophic nature of the genus *Listeria*, with the lowest detection rates found in slaughter rooms and the highest in low-temperature environments, such as boning rooms and cold rooms. In addition, 55 of the *Listeria* spp.-positive samples were obtained from deboning rooms, cold rooms, chilled half-carcasses, and meat cuts. Given this ability to thrive at low temperatures, the pathogen can be expected to persist for extended periods in both food products and refrigerated environments with temperatures as low as 4 °C (Zhou et al., 2024).

In meat cuts, the detection rates of *Listeria* spp. and *L. monocytogenes* were similar (Table 2), with the former being slightly higher. Nevertheless, all observed values are considered high. Notably, higher levels of *Listeria* spp. were found in unpackaged cuts, and *L. monocytogenes* was detected at equivalent levels in both unpackaged and vacuum-packaged cuts. These findings should serve as a warning to public health authorities and highlight the need for rigorous control measures in the industry.

Although similar in percentage, the detection rate of *Listeria* spp. in unpackaged cuts was higher than that in chilled half-carcasses. For *L. monocytogenes*, the frequency was higher in vacuum-packaged cuts. This may be related to the fact that half-carcasses were manually transported to the boning room, creating opportunities for recontamination via handlers or contact with surfaces such as conveyor belts, which showed high contamination levels (Sagawa et al., 2022).

The results for meat cuts in our study exceed those reported by Iglesias et al. (2017), who detected L. *monocytogenes* in 6 % (12/200) of meat products using PCR with ten different primer pairs. This discrepancy may be due to the specific primers used. On the other hand, our findings are lower than those of Palma et al. (2016), who reported *L. monocytogenes* in 24.4 % (11/45) of meat cuts analyzed by PCR. It is worth noting that the latter study included only 45 samples, which may account for the difference in results.

The detection percentages of *Listeria* spp. and *L. monocytogenes* in meat cuts and half-carcasses can be considered high. Notably, *Listeria* spp. was detected at particularly high levels in hot half-carcasses, exceeding the 13.3 % observed in chilled ones (Table 2). Furthermore, *Listeria* spp. was detected more frequently than the species under study. In contrast, Iglesias et al. (2017) reported *L. monocytogenes* frequencies ranging from 6.0 to 8.2 % in bovine carcasses at slaughterhouses in Brazil.

When comparing detection rates in hot half-carcasses (33.3 % for *Listeria* spp. and 16.7 % for *L. monocytogenes*) with those on skin rollers (13.3 %) (Table 2), it appears that contamination increased along the slaughter process. Since the microbiota of bovine carcass surfaces initially mirrors that of the skin (Ahmed and Al-Mahmood, 2023), the marked difference in contamination levels between half-carcasses and skin rollers suggests considerable cross-contamination, especially given that half-carcasses at room temperature were sampled at the end of the slaughter flowchart.

This contamination may result from contact with contaminated equipment and utensils, or from operational failures during slaughter, particularly during skinning and evisceration. As these microorganisms remain on carcass surfaces, they may also contaminate the environment and compromise the entire production process (Ovuru et al., 2024), as evidenced by the 15 % and 13.3 % detection rates of *Listeria* spp. and *L. monocytogenes*, respectively, in meat cuts from these slaughterhouses (Table 2).

Although not directly evaluated in this study, since personal and industrial hygiene practices were beyond its scope, the above propositions are supported by the extensive technical literature, which demonstrates that the primary sources of contamination and dissemination of microorganisms are considered to be cross-contamination involving animal skin, handlers, equipment, and the environment during

slaughter. The skin and intestinal contents are major contamination sources for bovine carcasses, and skinning and evisceration are identified as critical points in the slaughter process (Sagawa et al., 2022).

When comparing the detection rates of *Listeria* spp. in hot half-carcasses, a sharp decline is seen after chilling, as also observed with *L. monocytogenes*, although to a lesser extent. While the genus showed a 20 % decrease, the reduction for the species was only about 4 %. This significant decrease is likely attributable to the final washing of half-carcasses with potable water at the end of the slaughter flowchart, a step that may promote mechanical removal of microorganisms, since the establishments studied did not employ any additional decontamination procedures.

The reduction in the percentages above can also be partially explained by the surface drying of the half-carcasses and the application of low temperatures immediately after slaughter, particularly when cooling is more intense. These conditions reduce humidity and water activity (a*w*), thereby limiting the growth of contaminating microorganisms (Liang et al., 2022). Additionally, it is essential to highlight that the hot and refrigerated samples analyzed in the present study were not the same.

The lowest levels of *Listeria* spp. (10 %) and *L. monocytogenes* (10 %) were detected in the slaughter rooms (Table 2), with both being more frequently found on skin rollers. It is also worth noting that the percentages observed in drains were lower. These findings are justified by the fact that the slaughter room is continuously washed with water (often hot water or steam), throughout the slaughter process, which helps reduce contamination in drains. In contrast, the skin rollers are sanitized only after the slaughtering operations are completed.

Although it has been detected in half-carcasses and cuts, which are fresh meats rather than ready-to-eat products, typically the types of foods most associated with outbreaks (Ibarguren et al., 2022), these findings do not represent a lower risk or lesser importance. Fresh meat is commonly used as raw material for a wide variety of such products, including ready-to-eat raw kibbeh patties, hamburgers, and meatballs, among others. Furthermore, the frequent consumption of meat or meat products that are not sufficiently cooked is also noteworthy. These aspects are supported by the results of Cavalcanti et al. (2022), who reported the presence of *L. monocytogenes* in 14 % and 11 % of raw meat and ready-to-eat meat samples, respectively, from beef sold in Brazil between 2009 and 2019.

The results obtained in this study are compatible with those of Teixeira et al. (2020), who aimed to determine the frequency of *L. monocytogenes* occurrence at various stages of slaughter in bovine slaughterhouses certified for international trade in the state of Mato Grosso, Brazil, using the PCR technique. They observed a prevalence of 12 % in the samples analyzed. Although the isolated strains were not resistant to the antibiotics tested, they exhibited genes and resistance profiles to sanitizers such as hypochlorite.

4.2. Efficiency of the primer pairs used to detect L. monocytogenes in the sources under study

The total number of samples positive for *L. monocytogenes* identified in this study was only made possible by the combined use of the two primer pairs, despite the low efficiency of the LL5/LL6 pair.

The results obtained revealed a high performance of the LM1/LM2 pair for detecting *L. monocytogenes* in the total samples analyzed, as its use enabled the identification of 92.4 % of the positive samples, whereas the LL5/LL6 pair demonstrated efficiency that can be considered low (Table 3). Despite this, four additional positive samples were identified with the LL5/LL6 pair that were not detected with the LM1/LM2 pair (Box 1), increasing the total number of positive samples from 49 to 53 and consequently raising the detection percentage to 14.7 % (Table 3), compared to the 13.6 % that would have been found using only the LM1/LM2 pair.

Although this appears to be a small difference, it cannot be overlooked given the need to reduce the occurrence of false-negative results. This is particularly relevant for environmental samples from processing plants, and especially for food, considering that foodborne transmission of *L. monocytogenes* is one of the main routes of human infection, in addition to the severity of the disease and the high lethality rates reported (Chen et al., 2021).

Regarding the detection sources, the best results for the LM1/LM2 pair were observed in slaughter rooms, boning rooms and cold rooms (100 %, 94.7 %, and 91.7 %, respectively) (Table 3). It is noteworthy that all the positive samples in the slaughter rooms were identified only through this pair. At the sampled sites, all positive samples were found in drains, rollers and conveyors in boning rooms; unpackaged meat cuts; evaporators in cold rooms; chilled half-carcasses; drains in slaughter rooms; and skin rollers.

Slightly lower efficiency of the LM1/LM2 pair was observed in meat cuts and half-carcasses (87.5 %) (Table 3), and its lowest performance was recorded in boning room evaporators and vacuum-packed cuts (75 %) (Table 3).

It is possible that the reduced performance of these primers is partly due to the presence of cells with a higher degree of injury (Milton et al., 2023), since in sources such as half-carcasses and meat cuts, a higher bacterial population is expected due to the nature of the substrate. Another factor to consider, particularly in the case of evaporators, is the possibility of an initially low-level contamination, combined with the sample collection method by swabbing.

An analysis of the results presented in Table 3 shows that the LL5/LL6 primers were unsatisfactory for detecting *L. monocytogenes* in both the individual sources and the overall sample. The highest detection rate was observed in cold rooms (66.7 %), while the efficiency was significantly lower in other sources, especially in samples from slaughter rooms. In sites such as boning room evaporators, vacuum-packed meat cuts, drains, and cold room evaporators, performance was notably better, identifying between 60 % and 75 % of the positives (Table 3). Conversely, no amplification signals were obtained for unpackaged meat cuts and slaughter room drains.

These findings further emphasize the importance of using more than one primer pair for detecting the bacterium in the evaluated sources and imply a need to assess additional pairs to improve the sensitivity of the technique. These points are well supported in the literature, as several authors have discussed the difficulties in detecting microorganisms in food using the PCR technique (Golsteyn Thomas et al., 1991; Milton et al., 2023; Wang et al., 2021).

The LL5/LL6 primers appear to have performed better in sources where the bacterium was less likely to suffer injurious effects or where contamination levels were presumably higher, such as in boning room evaporators, cold room evaporators and drains, and vacuum-packed meat cuts. These conditions likely favored bacterial growth during sample enrichment, increasing the number of existing cells and compensating for the apparently low detection sensitivity of these primers in other sources.

The results suggest that the LL5/LL6 pair was more suitable for detecting *L. monocytogenes* in samples from low-temperature environments, where there was a lower likelihood of severe cell injury. This interpretation is supported by the data in Table 2. Moreover, this primer pair was not suitable for use in sources with high temperatures and/or low bacterial counts. It is also worth noting that in many instances where LL5/LL6 performed best, the LM1/LM2 pair showed poorer performance, as observed in samples from cold room drains and boning room evaporators.

5. Conclusion

Listeria spp. and *L. monocytogenes* are widespread in the sources and sites studied, with levels considered high, at 19.7 % and 14.7 %, respectively. The combination of the two primer pairs increased detection rates and was necessary to identify all the positive samples in this

study. The LL5/LL6 pair did not prove satisfactory for detecting *L. monocytogenes* in the sources analyzed, and additional primers should be evaluated for detecting the bacterium in these contexts. The presence of *Listeria* spp. and *L. monocytogenes* in samples from slaughterhouses in different Brazilian states reveals the wide distribution of these microorganisms and should serve as a warning to the relevant health authorities.

CRediT authorship contribution statement

Leonardo França: Writing – original draft, Formal analysis, Conceptualization. **Ursula Nunes Rauecker:** Writing – original draft, Investigation, Conceptualization. **Daniel Lucino Silva dos Santos:** Writing – review & editing, Writing – original draft, Data curation. **Naara Aparecida Almeida:** Writing – original draft, Data curation. **Clarice Gebara:** Writing – review & editing, Writing – original draft. **Cristiano Sales Prado:** Writing – review & editing, Data curation. **Valéria Quintana Cavicchioli:** Writing – original draft, Investigation. **Moacir Evandro Lage:** Investigation, Data curation. **Francine Oliveira Duarte:** Writing – original draft. **Cíntia Silva Minafra e Rezende:** Writing – review & editing, Supervision, Project administration. **Ana Maria de Souza Almeida:** Writing – review & editing, Writing – original draft. **Iolanda Aparecida Nunes:** Writing – review & editing, Supervision, Project administration.

Ethical statement

This article does not contain any studies with human participants or animals performed by any of the authors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. no conflict of interest.

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