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Quantitative microbiological risk assessment for the occurrence of listeriosis in Brazil due to the consumption of milk processed by pasteurization or thermosonication

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ABSTRACT

This study aimed to estimate the risk of listeriosis from the consumption of pasteurized milk in Brazil, comparing conventional treatment with the technology of thermosonication. The Quantitative Microbiological Risk Assessment (QMRA) model was developed, covering the entire milk production chain, from milking to the moment of consumption. In general, higher risks were observed in association with higher initial concentrations of the pathogen and the vulnerable population. The highest risk predicted (3.67×10^{-5}) was related to the scenario considering the initial concentration range of *L. monocytogenes* between 4 and 6 log CFU/mL, with conventional treatment and considering the vulnerable population, resulting in one case of listeriosis every 27,248 servings. When considering thermosonication treatment, lower risks have been predicted. The scenario analysis indicated that the steps related to storage conditions in retail and at the consumer's home (post-processing steps) are the most influential in the associated risk, in all scenarios. The predictive parameters of inactivation related to the applied treatment also have a considerable influence on the risk. The results point to the influence of the stages of the dairy production chain and the thermosonication treatment applied in the food safety of milk, subsidizing information for industrial application and for regulatory agencies.

1. Introduction

Listeria monocytogenes is a Gram-positive microorganism considered a pathogen to humans, causing the disease called listeriosis. In immunocompromised individuals (such as the elderly and pregnant women) it can cause more serious problems, such as meningitis, miscarriage and septicemia, generating a high mortality rate (Lecuit, 2020).

Dairy products are one of the food groups most associated with contamination by *L. monocytogenes*. Its production chain has several critical points where contamination can occur, especially contaminated silage, which will lead to the contamination of the pathogen through feces and milk, thus perpetuating the transmission cycles of the microorganism and generating considerable bacterial loads in the milking environment (Amado et al., 2016).

Despite being sensitive to heat treatments in general, such as pasteurization, *L. monocytogenes* is resistant to certain adverse environmental conditions, such as low pH and low temperatures, characterizing it as a psychrotolerant microorganism. Thus, controlling the growth of this pathogen in the food production chain is a challenge, especially considering products that need a cold chain, such as dairy products in general (Leclair et al., 2019; Silva et al., 2021).

The application of high temperatures in foods can cause negative changes related to sensory, nutritional and technological properties, especially in dairy products. Thus, conventional heat treatments, such as pasteurization, have given way to so-called emerging technologies, such as ultrasound, which is capable of generating products with superior quality, due to the lower temperatures and processing times applied, with proven efficiency in microbiological safety. Thermosonication, which is based on the joint application of ultrasound and temperature, has shown satisfactory results in all aspects when applied to dairy products (Oliveira et al., 2022).

A scientific approach that estimates the risk associated with a

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foodborne pathogen and food combination is the quantitative microbial risk assessment (QMRA), which can provide valuable data regarding the risks related to a particular pathogen in a given population when a given food is consumed. A QMRA model is based on the numerical construction of all stages of the food production chain, applying probability distributions to obtain more reliable estimates (Campagnollo et al., 2018; Dogan et al., 2020; Ramos et al., 2021).

Thus, this study aimed to build a QMRA model to estimate the risk of listeriosis in Brazil from the consumption of milk treated by pasteurization, compared to the product treated by thermosonication. To better verify the influence of processing steps on the final risk, different scenarios were evaluated considering different initial concentrations of *L. monocytogenes* in raw milk.

2. Material and methods

A QMRA model was built to quantify the risk associated with the occurrence of listeriosis through the consumption of milk treated by conventional pasteurization or by thermosonication. The construction of the model considered the entire production chain, from milking to the moment of consumption, and was divided into four modules: raw milk module, processing module, retail/domestic module, and consumption module. The model construction was the same for both treatments considered, except for the processing module, where the predictive parameters of microbial inactivation differ. Any possible recontamination after processing was disregarded.

Table 1
Raw milk module.

Notation	Description	Value	Unit	Source
P_{LM}	Prevalence of *L. monocytogenes* in raw milk in Brazil	Beta (32,401)	%	Figueiredo (2000); Catão and Ceballos (2001); Camargo (2010); Cavalli et al. (2016)
$LM_{initial}$	Initial contamination level of *L. monocytogenes* in raw milk	If $P_{LM} > 0$; $LM_{initial}$ = Pert (0.1,1,2); Pert (2,3,4); or Pert (4,5,6)	Log CFU/mL	Latorre et al. (2011); Meyer-Broseta et al. (2003); Dogan et al. (2020)
t_{raw}	Time until refrigerated storage	Normal (0.083,0.0417, Truncate 0,0.17)	days	Brasil (2019)
TP_{raw}	Storage temperature until refrigerated storage	Uniform (25,30)	°C	Assumption
T_{min}	Minimum growth temperature of *L. monocytogenes* in milk	Uniform (−2,−1)	°C	WHO and FAO (2004)
μ_5	Growth rate of *L. monocytogenes* in milk at 5 °C	Uniform (0.092, 0.434)	Log CFU/day	WHO and FAO (2004)
μ_{raw}	Growth rate of *L. monocytogenes* at storage temperature	$\mu_5[(TP_{distr} - T_{min})/(5 - T_{min})]^2$	Log CFU/day	WHO and FAO (2004)
LMG_{raw}	*L. monocytogenes* growth during storage until refrigerated storage	$\mu_{raw} * t_{raw}$	Log CFU/mL	Calculated
N_{max}	Growth limit ($T >$ 7 °C)	8	Log CFU/mL	WHO and FAO (2004)
LM_{raw}	*L. monocytogenes* concentration in raw milk before pasteurization	min [($LM_{initial}$ + LMG_{raw}), N_{max}]	Log CFU/mL	Calculated

2.1. Raw milk module

The first module (Table 1) aims to obtain the concentration of *L. monocytogenes* in milk immediately before the processing step. Initially, the prevalence of the pathogen in Brazilian milk was considered, according to four studies that performed this determination in raw milk (Camargo, 2010; Catão and Ceballos, 2001; Cavalli et al., 2016; Figueiredo, 2000). Thus, the prevalence was estimated using a beta distribution based on the presence of *L. monocytogenes* in 32 of the 401 raw milk samples analyzed. From simulation data, it was observed that the prevalence did not reach zero, according to the probability distribution, oscillating between 0.033 and 0.136. Thus, the hypothesis of null prevalence was not considered in the construction of the model.

To estimate the concentration of *L. monocytogenes* in raw milk, it was observed that the national studies did not perform the quantification, they only indicated the prevalence of the pathogen. This can be justified based on the history of Brazilian legislation regarding food microbiological standards. For two decades, the current legislation determined the absence of *L. monocytogenes* in certain food groups, a situation that was changed with the entry into force of the new legislation, in 2019, which started to tolerate certain concentration limits, depending on the food group (Brasil, 2019). Thus, usual concentrations in raw milk were observed for other locations, obtaining values of up to 6 log CFU/mL (Dogan et al., 2020; Latorre et al., 2011; Meyer-Broseta et al., 2003). For the construction of the model, scenarios were defined varying the concentration of *L. monocytogenes* in raw milk. The 1 log scenario was defined as a Pert distribution of most likely value as 1 log CFU/mL, and minimum and maximum values of 0.1 and 2 log CFU/mL, respectively. Scenarios 3 and 5 log followed the same logic, with Pert (2,3,4) and Pert (4,5,6) distributions, respectively. In this model, it was assumed that the only source of pathogen contamination in the production chain was directly raw milk, disregarding any possible contamination after processing.

Brazilian legislation determines that the maximum period for raw milk to be refrigerated is 3 h, from the moment of milking (Brasil, 2018). Thus, the QMRA model developed considers a period at room temperature (25–30 °C) in which the milk is kept and there is the multiplication of microorganisms. Based on the maximum period of 3 h, this step was represented as a normal distribution with a mean of 120 min and a deviation of 60 min and assuming a temperature represented by a uniform distribution between 25 and 30 °C (room temperature). To quantify growth, a predictive model was used where the growth rate during the storage period before cooling was represented by Eq. (1) (WHO and FAO, 2004):

$$\mu_{raw} = \mu_5 * \left((T_{raw} - T_{min}) / (5 - T_{min}) \right) 2 \quad (1)$$

where μ_{raw} is the growth rate of *L. monocytogenes* in raw milk; μ_s is the growth rate of *L. monocytogenes* at 5 °C; T_{raw} is the storage temperature until refrigeration and T_{min} is the minimum growth temperature of *L. monocytogenes* in milk.

Considering the growth limit of 8 log CFU/mL of *L. monocytogenes* at temperatures above 7 °C (WHO and FAO, 2004), the model predicts the choice of the lowest value between this limit and the concentration value obtained by applying the predictive model. According to the mathematical details shown in Table 1, at the end of the raw milk module, the concentration of *L. monocytogenes* immediately before processing is obtained.

2.2. Processing module

In the processing module (Table 2), the predictive parameters of inactivation of *Listeria innocua* (surrogate of *L. monocytogenes*) in milk treated by conventional pasteurization (65 °C/30 min) or by thermosonication (400 W, 24 kHz, 108 μm: 2.57 W/cm^2, 30 min; Bermúdez-

Table 2
Processing module.

Notation	Description	Value pasteurization	Value thermosonication	Unit	Source
t_{treat}	Time of treatment of milk	30		min	Bermúdez-Aguirre et al. (2009)
δ	Delta parameter of Weibull model	Normal (0.016,0.0016)	Normal (2.9,0.29)	min^{-1}	Bermúdez-Aguirre et al. (2009)
p	p parameter of Weibull model	Normal (1.73,0.173)	Normal (0.21, 0.021)	–	Bermúdez-Aguirre et al. (2009)
LM_{treat}	*L. monocytogenes* concentration in treated milk	LM_{raw} - (δ)*(t^{p}_{treat})		Log CFU/mL	Calculated

Aguirre et al., 2009) are described, and applied in the mathematical model of Weibull, according to Eq. (2):

$$LM_{treat} = LM_{raw} - \left(\delta^{*} t^{p}_{treat}\right) \quad (2)$$

where LM_{treat} is the concentration of *L. monocytogenes* in the milk at the end of processing (log CFU/mL); LM_{raw} is the concentration of *L. monocytogenes* in milk before processing (log CFU/mL); δ is the parameter of the Weibull model related to the inactivation rate; t_{treat} is the processing time and p is the parameter related to the curvature of the inactivation model.

In the construction of the model, the mathematical parameters of the predictive model were inserted as normal distributions with a standard deviation estimated at 10 %, since the original study does not present values of standard deviation.

2.3. Retail/domestic module

In the retail/domestic module (Table 3), the stages that the treated milk goes through after processing until the moment of consumption were considered. Initially, the transport conditions of the product from the industry to the point of sale were considered, represented by Pert distributions with the values of time (average of 3.7 h) and temperature (average of 6.7 °C) of transport obtained from a study carried out by Koutsoumanis et al. (2010), considering that there is no such study carried out in Brazil.

At the point of sale, the product's permanence time is estimated by a log-normal distribution with a mean of 20 h and a standard deviation of the same value, truncated at 96 h, considering that the product's shelf life is on average 5 days, and the product is usually removed from the point of sale one day before the expiration date (Koutsoumanis et al., 2010). The storage temperature in refrigerators at Brazilian points of sale is estimated to be between 6.4 and 15.5 °C, as indicated by Campagnollo et al. (2018).

Domestic storage time was represented as a Pert distribution with a most likely value of 48 h, ranging from 24 to 72 h, considering that the consumer purchased the product at the point of sale at least two days before the expiration date. The storage temperature in Brazilian domestic refrigerators is represented by a uniform distribution with values between 3.04 and 10.82 °C (Silva et al., 2008).

From the storage times and temperatures described in the transport, retail and domestic stages, the growth of *L. monocytogenes* in the treated product was estimated using a predictive growth model, applying each stage to Eq. (1) (WHO and FAO, 2004). At the end of the retail/domestic module, the concentration of *L. monocytogenes* in milk at the time of consumption is obtained, as outlined in Table 3.

2.4. Consumption module

The consumption module is shown in Table 4. The serving size is represented by a Pert distribution with the most likely value of 350 mL, according to a study carried out by Ntuli et al. (2018), since there is no record of this type of Brazilian source.

The dose-response function to estimate the risk of listeriosis from consumption of treated milk was calculated using an exponential model developed by Buchanan et al. (1997). The parameter r, which represents the probability associated with a single cell causing listeriosis in an individual, was used for the general population and for the vulnerable population, separately (WHO and FAO, 2004), as described in Table 4.

Thus, at the end of the model, outputs are obtained for the general and vulnerable populations, for milk treated by pasteurization or thermosonication, in each scenario of initial concentration of *L. monocytogenes* in raw milk. The risk per serving for each population group evaluated is obtained by considering the initial prevalence of *L. monocytogenes* in raw milk (Table 4). As there is no data on the consumption of pasteurized milk in Brazil, demographics and consumption data of the population are not considered in this model, and the outputs are the risk of listeriosis per serving (probability of occurrence of illness by the consumption of one serving) and the number servings needed for a case of listeriosis to occur.

Table 3
Retail/domestic module.

Notation	Description	Value	Unit	Source
t_{distr}	Transport time during distribution	Pert (0.0083,0.1542,0.425)	days	Koutsoumanis et al. (2010)
T_{distr}	Temperature during distribution	Pert (3.6,6.7,10.9)	°C	Koutsoumanis et al. (2010)
t_{retail}	Storage time at retail	Log normal (0.833; 0.833; truncate [4])	days	Koutsoumanis et al. (2010)
T_{retail}	Temperature during storage at retail	Uniform (6.4,15.5)	°C	INMETRO (2006)
$t_{domestic}$	Storage time at home	Pert (1,2,3)	days	Assumption
$T_{domestic}$	Temperature at domestic storage	Uniform (3.04,10.82)	°C	Silva et al. (2008)
μ_{distr}	*L. monocytogenes* growth rate at temperature in distribution	$\mu_5[(T_{distr} - T_{min})/(5 - T_{min})]^2$	Log CFU/day	WHO and FAO (2004)
LMG_{distr}	*L. monocytogenes* growth during distribution	$\mu_{distr} * t_{distr}$	Log CFU	Calculated
μ_{retail}	*L. monocytogenes* growth rate at temperature at retail	$\mu_5[(T_{retail} - T_{min})/(5 - T_{min})]^2$	Log CFU/day	WHO and FAO (2004)
LMG_{retail}	*L. monocytogenes* growth during retail storage	$\mu_{retail} * t_{retail}$	Log CFU	Calculated
$\mu_{domestic}$	*L. monocytogenes* growth rate at temperature in domestic storage	$[\mu_5(T_{domestic} - T_{min})/(5 - T_{min})]^2$	Log CFU/day	WHO and FAO (2004)
$LMG_{domestic}$	*L. monocytogenes* growth during domestic storage	$\mu_{domestic} * t_{domestic}$	Log CFU	Calculated
LM_{cons}	*L. monocytogenes* concentration at the consumption	min $[(LM_{treat} + LMG_{distr} + LMG_{retail} + LMG_{domestic}), N_{max}]$	Log CFU/mL	Calculated

Table 4
Consumption module.

Notation	Description	Value	Unit	Source
SS	Serving size	Pert (200,350,500)	mL	Ntuli et al. (2018)
$LM_{serving}$	*L. monocytogenes* concentration in a serving	LM_{cons} * SS	Log CFU/ serving	Calculated
r_{gen}	Parameter r for dose response for general population	2.37×10^{-14}	–	WHO and FAO (2004)
r_{vuln}	Parameter r for dose response for vulnerable population	1.06×10^{-12}	–	WHO and FAO (2004)
$Risk_{gen}$	Risk of listeriosis per serving for general population	(1 - Exp ($-r_{gen}$*$LM_{serving}$))* P_{LM}	–	Buchanan et al. (1997)
$Risk_{vuln}$	Risk of listeriosis per serving for vulnerable population	(1 - Exp ($-r_{vuln}$*$LM_{serving}$))* P_{LM}	–	Buchanan et al. (1997)
N_{sgen}	Number of servings for 1 case of listeriosis for general population	$1/Risk_{gen}$	servings/ case	Calculated
N_{svuln}	Number of servings for 1 case of listeriosis for vulnerable population	$1/Risk_{vuln}$	servings/ case	Calculated

2.5. Evaluated scenarios and Monte Carlo simulation

As described in the raw milk module, scenarios were evaluated considering ranges of initial concentrations of *L. monocytogenes* in raw milk (scenarios 1 log, 3 log and 5 log), for each type of processing applied, and considering the general and vulnerable population.

To obtain the outputs, Monte Carlo simulation was used, which is characterized by the substitution of points for probability distributions in the inputs. The simulation was performed in @Risk software version 8.2 (Palisade, United States), using 100,000 iterations. A large number of iterations reflect a greater coverage of the statistical distribution, bringing more accurate results (Ramos et al., 2021).

3. Results and discussion

The presence of *L. monocytogenes* in raw milk can be explained by possible contact with environmental sources such as water, animal feeds, feces, or soil. Bovine mastitis can also be a source of the pathogen, which may be the causative agent of this infection. Furthermore, one must consider the psychrophilic nature of *L. monocytogenes*, which can multiply at refrigeration temperatures, and its ability to form biofilms, making this species relevant in the microbiological control of dairy products (Campagnollo et al., 2018; McIntyre et al., 2015). In this model, the presence of *L. monocytogenes* only from raw milk origin was considered, disregarding any contamination during processing or subsequent step.

Table 5 shows the risks of occurrence of listeriosis due to the consumption of pasteurized milk in Brazil, in the different scenarios evaluated, obtained by Monte Carlo simulation. Monte Carlo simulation allows uncertainty and variability to be incorporated into the model inputs (Ramos et al., 2021). Scenarios were evaluated with different ranges of initial concentrations of *L. monocytogenes* in raw milk (scenarios 1, 3 and 5 log) treated by conventional pasteurization or thermosonication, for the general or vulnerable population.

In both treatments, the greater the initial pathogen concentration range, the greater the risk associated with the occurrence of listeriosis. Furthermore, the risk of listeriosis per serving associated with the vulnerable population is always higher when compared to the general population in the same scenario. In scenarios associated with milk treated by conventional pasteurization, the mean risk ranged between 2.70×10^{-8} and 8.32×10^{-7} for the general population, and between 1.19×10^{-6} and 3.67×10^{-5} for the vulnerable population. In the scenarios associated with thermosonicated milk, the mean risk ranged between 5.35×10^{-9} and 2.11×10^{-7} for the general population, and between 2.36×10^{-7} and 9.28×10^{-6} for the vulnerable population. Among all scenarios, the highest average risk was predicted in the 5 log scenario, treated by conventional pasteurization, for the vulnerable population (3.67×10^{-5}).

Evaluating the average risks associated with the general and vulnerable populations (Table 5), it is noted that in both treatments, there is a large difference in the predicted risks, where the risk associated with the general population corresponds, on average, to only 2.3 % of the risk associated with the vulnerable population.

The vulnerable population comprises immunodeficient consumers, such as young children, pregnant women and the elderly, who are more susceptible to foodborne illness. Vulnerability differs between different groups, with the elderly group being more exposed to foodborne diseases due to several factors, such as decreased immunity due to age, changes in the gastrointestinal tract and a sedentary lifestyle. With regard specifically to listeriosis, attention is drawn to groups of pregnant

Table 5
Risks associated with the scenarios evaluated.

		CONV			TS		
		1 log	3 log	5 log	1 log	3 log	5 log
Risk general	Mean	2.70×10^{-8}	1.44×10^{-7}	8.32×10^{-7}	5.35×10^{-9}	4.39×10^{-8}	2.11×10^{-7}
	SD	1.12×10^{-6}	2.68×10^{-6}	6.43×10^{-6}	5.66×10^{-7}	1.47×10^{-6}	3.22×10^{-6}
	Minimum	0	0	0	0	0	5.55×10^{-17}
	5th percentile	0	0	0	0	7.93×10^{-16}	7.39×10^{-14}
	Median	1.43×10^{-15}	1.49×10^{-13}	1.44×10^{-11}	8.549×10^{-16}	8.28×10^{-14}	8.11×10^{-12}
	95th percentile	4.38×10^{-11}	4.51×10^{-9}	4.64×10^{-7}	8.17×10^{-13}	8.39×10^{-11}	8.60×10^{-9}
	Maximum	8.16×10^{-5}	1.03×10^{-4}	1.19×10^{-4}	8.65×10^{-5}	9.53×10^{-5}	9.69×10^{-5}
Risk vulnerable	Mean	1.19×10^{-6}	6.34×10^{-6}	3.67×10^{-5}	2.36×10^{-7}	1.94×10^{-6}	9.28×10^{-6}
	SD	4.92×10^{-5}	1.18×10^{-4}	2.83×10^{-4}	2.48×10^{-5}	6.46×10^{-5}	1.42×10^{-4}
	Minimum	0	0	0	0	9.91×10^{-17}	2.38×10^{-15}
	5th percentile	0	0	0	3.77×10^{-16}	3.54×10^{-14}	3.31×10^{-12}
	Median	6.41×10^{-14}	6.67×10^{-12}	6.45×10^{-10}	3.80×10^{-14}	3.71×10^{-12}	3.63×10^{-10}
	95th percentile	1.96×10^{-9}	2.02×10^{-7}	2.08×10^{-5}	3.65×10^{-11}	3.75×10^{-9}	3.85×10^{-7}
	Maximum	3.60×10^{-3}	4.50×10^{-3}	5.18×10^{-3}	3.79×10^{-3}	4.17×10^{-3}	4.24×10^{-3}

SD: Standard deviation. CONV: Conventional treatment. TS: Treatment by thermosonication. 1 log: scenario characterized by the initial concentration of *L. monocytogenes* represented by Pert distribution (0.1,1,2), in log CFU/mL. 3 log: scenario characterized by the initial concentration of *L. monocytogenes* represented by Pert distribution (2,3,4), in log CFU/mL. 5 log: scenario characterized by the initial concentration of *L. monocytogenes* represented by Pert distribution (4,5,6), in log CFU/mL.

and diabetic and immunodeficient women, who are 17 and 25 times more likely to contract listeriosis, respectively, when compared to the general population (Dumitrașcu et al., 2020; Evans and Redmond, 2015; Feng et al., 2016).

Regarding the treatments used in milk processing, the risks associated with the product treated by thermosonication were lower in all simulated scenarios. Ultrasound technology is consolidated with regard to microbial inactivation, reaching high levels of effectiveness due to the acoustic cavitation mechanism. The microbubbles generated by cavitation and their subsequent collapses cause turbulence in the food, which is capable of breaking the cell membrane and causing leakage of the cellular components of the microorganism, in addition to generating free radicals that interact with cellular DNA. The joint application of temperature makes the treatment even more effective (Bhargava et al., 2021; Guimarães et al., 2021). Thermosonication technology has been shown to be highly effective in recent studies, especially when applied to dairy products such as cheeses, dairy drinks and raw milk (Niamah, 2019; Oliveira et al., 2022; Scudino et al., 2020).

The greater microbial inactivation indicated by the mathematical parameters of the Weibull inactivation model justifies the difference found in the associated risk since the treatment by emerging technology generates greater inactivation in the processing step. The Weibull model has been frequently used to predict the inactivation of various pathogens in food matrices, especially associated with the study of new technologies (Liu et al., 2019; Lobacz et al., 2020; Pereira et al., 2020).

Based on the risk values obtained, it is possible to estimate that a case of listeriosis will occur at every given number of servings, performing the inverse of the numerical value of the average risk for each scenario (Table 6). Analysis of these data provides greater clarity in comparing the associated risks, where a greater number of servings required indicate a lower risk of disease occurrence. Attention is drawn to the large number of servings to result in one case of listeriosis in the 1 log scenarios for the general population (in the order of millions), indicating the lowest predicted risks, and the low number of servings in the 5 log scenario, where in every 27,248 servings of the product treated by pasteurization for the vulnerable population there would be a case of listeriosis.

Comparing the risks associated with listeriosis from the consumption of dairy products from other studies is difficult due to the complexity, particularities and considerations of each QMRA model considered. Recent studies have evaluated the risk of listeriosis associated with different types of cheese in different locations. Campagnollo et al. (2018) considered a scenario with milk with a concentration of 6 log CFU/mL of *L. monocytogenes* for the production of semi-hard cheese and milk with a concentration of 1 log CFU/mL for the production of fresh soft cheese, where they obtained risks in the order of 10^{-5} and 10^{-1}, respectively, for the general population. In this work, the authors considered the contamination of milk after the pasteurization process, disregarding the stage of pathogen prevalence in the raw material and pre-processing storage and transport conditions.

Condoleo et al. (2017) performed a QMRA study associating the risk of listeriosis with the consumption of cheese made from raw sheep's milk in Italy, obtaining values in the order of 10^{-10} and 10^{-12}. However, in this study, several assumptions were adopted that directly impact the accounting of the concentration of the microorganism during the production chain, such as assuming zero pre-processing storage time and that there was no microbial growth during the domestic and retail storage stages. In addition, attention should be paid to the large differences related to the origin of the milk, where sheep's milk has much lower prevalence data and initial pathogen concentration (Condoleo et al., 2017).

Fig. 1 displays the Tornado graphs of the scenario analysis for 3 and 5 log scenarios. Scenario analysis establishes a relationship between the input and output values of the QMRA model, represented by Tornado graphs, where each input parameter (or step) of the model is represented by a bar, and the length of each bar signals the influence of the parameter on the model's final output (Ramos et al., 2021).

In all scenarios, storage time at retail was the parameter with the greatest influence on the risk of listeriosis, and the temperature associated with this step is one of the four most influential parameters. The highlight of the retail storage time compared to storage in transport or at the consumer's home is due to the higher temperature range attributed to this stage (Table 3), according to national data, allowing greater growth of the pathogen as a function of the binomial time/temperature. It is visible that the parameters associated with the retail/domestic module are the most influential in all scenarios, indicating the importance of controlling these steps by retailers and consumers. The stages where storage occurs for considerable periods of time are of great influence, especially due to the relationship between the slower metabolism observed in psychrotrophic microorganisms, where a longer time interval is necessary for their multiplication at refrigeration temperatures.

Predictive inactivation parameters also have a considerable influence on risk compared to other parameters (Fig. 1). It is observed that in the scenarios considering the thermosonication treatment there is a smaller influence of these parameters on the risk when compared to the conventional treatment. This can be justified by the fact that the thermosonication treatment presents parameters that characterize a faster microbial inactivation, where an eventual variation in the values would present a smaller variation in the behavior of the curve, causing a smaller impact on the associated risk.

It is noteworthy that the prevalence of *L. monocytogenes* in Brazilian raw milk exerts little influence on the risk of disease, in all scenarios evaluated, especially in the scenarios of higher initial concentration. Viewed from the product's production chain until the moment of consumption, the large number of steps that allow microbial development ends up prevailing with regard to the numerical contribution of the associated risk. Overall, the initial concentration of *L. monocytogenes* in raw milk has a greater influence than its prevalence, but less than the storage conditions along the production chain and the predictive parameters (Fig. 1).

4. Conclusion

The QMRA model developed made it possible to verify considerable risks of listeriosis related to the consumption of pasteurized milk in Brazil, based on national data and particularities inherent to the local production chain. From the results, it is possible to verify the importance of the production process, from the adoption of good milking practices, to the moment of consumption. The factors that were most influential in

Table 6
Number of servings required for a case of listeriosis to occur, in each scenario (1/risk).

	CONV			TS		
	1 log	3 log	5 log	1 log	3 log	5 log
General	37,037,037	6,944,444	1,201,923	186,915,888	22,779,043	4,739,336
Vulnerable	840,336	157,729	27,248	4,237,288	515,464	107,759

CONV: Conventional treatment. TS: Treatment by thermosonication. 1 log: scenario characterized by the initial concentration of *L. monocytogenes* represented by Pert distribution (0.1,1,2), in log CFU/mL. 3 log: scenario characterized by the initial concentration of *L. monocytogenes* represented by Pert distribution (2,3,4), in log CFU/mL. 5 log: scenario characterized by the initial concentration of *L. monocytogenes* represented by Pert distribution (4,5,6), in log CFU/mL.

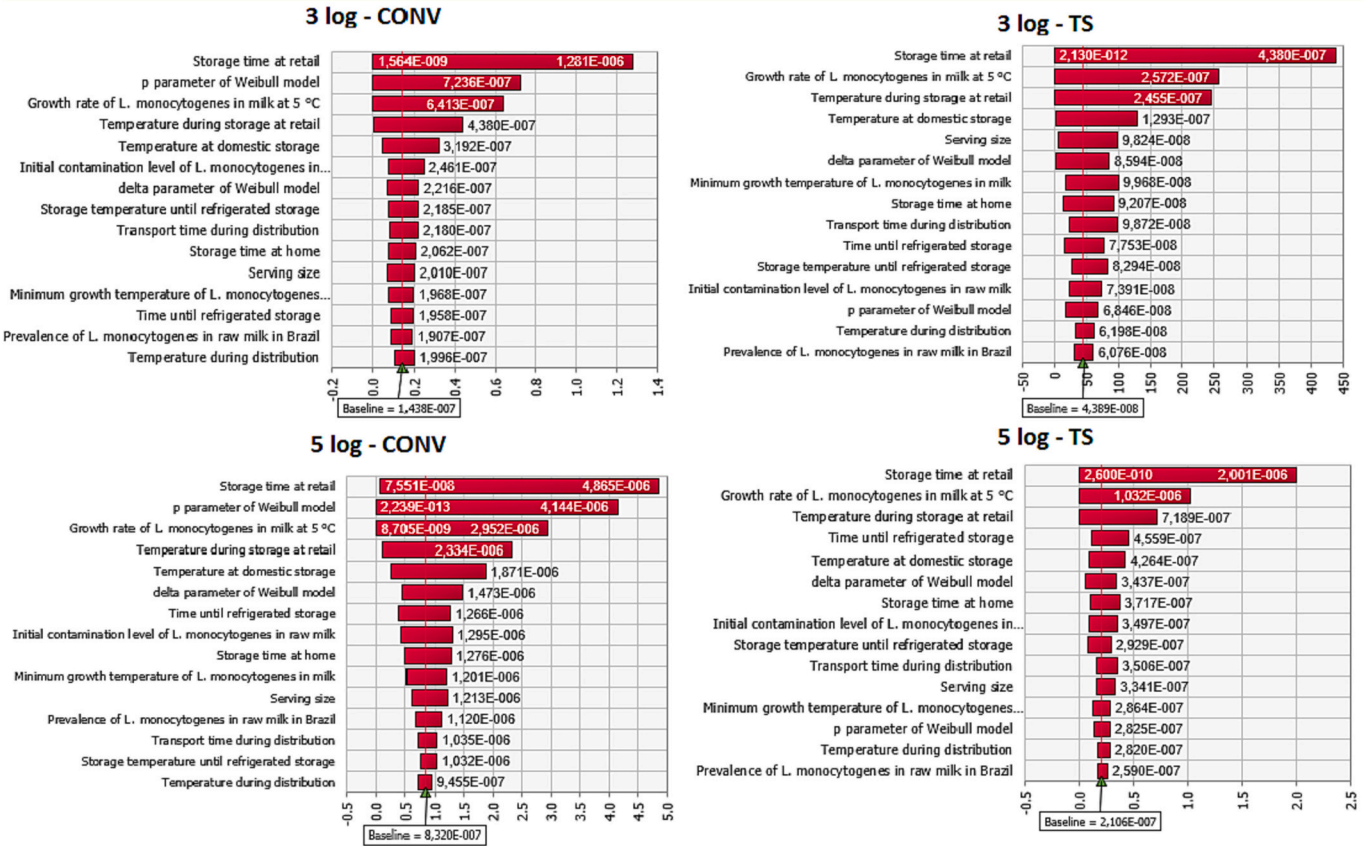

Fig. 1. Scenario analysis, where Tornado Graphs indicate which inputs contribute the most to risk. CONV: Conventional treatment. TS: Treatment by thermosonication. 3 log: scenario characterized by the initial concentration of *L. monocytogenes* represented by Pert distribution (2,3,4), in log CFU/mL. 5 log: scenario characterized by the initial concentration of *L. monocytogenes* represented by Pert distribution (4,5,6), in log CFU/mL. The displayed values correspond to the range of variation of each parameter. The triangles and the value indicated in the rectangles correspond to the average risk value in each scenario.

the associated risk were the storage and transport conditions related to the retail and domestic stages, calling attention to greater temperature control and storage time in these stages.

The treatment of milk by thermosonication generated lower risks associated with the occurrence of listeriosis, reinforcing the effectiveness of this emerging technology about microbiological safety. More studies are needed to develop QMRA models in dairy products, addressing the application of other emerging technologies and their impact on other pathogens, expanding the numerical database that helps regulatory agencies in the scope of risk analysis.

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.

Data availability

Data will be made available on request.

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