International Journal of Food Microbiology 379 (2022) 109843

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

A quantitative risk assessment of *Listeria monocytogenes* from prevalence and concentration data: Application to a traditional ready to eat (RTE) meat product

Chrystalleni Hadjicharalambous [a,b,d,*], Luca Grispoldi [b], Thanos Chalias [b,d], Beniamino Cenci-Goga [b,c,**]

[a] Department of Chemistry, University of Crete, Heraklion 70013, Greece
[b] Medicina Veterinaria, Laboratorio di Ispezione degli Alimenti di Origine Animale, Universita degli Studi di Perugia, 06126 Perugia, Italy
[c] Faculty of Veterinary Science, Department of Paraclinical Sciences, University of Pretoria, Onderstepoort 0110, South Africa
[d] European Food Safety Authority, EU-FORA Programme, Parma, 43126, Italy

ARTICLE INFO

Keywords:
Italian head cheese
Listeriosis
Food safety
Microbiological risk

ABSTRACT

Ready to Eat (RTE) cooked meat products are among the most consumed RTE food subcategories in the EU/EEA. They are also associated with the highest number of identified listeriosis cases per year (>850), thus posing a public health risk especially among the susceptible population. This study estimated the risk of listeriosis from Italian head cheese (Coppa di Testa) consumption using a Quantitative Microbiological Risk Assessment (QMRA) based on data of prevalence and starting concentrations of *Listeria monocytogenes* in the product during a 3-year period (n = 1568). A consumer survey (n = 162) was conducted to provide information on domestic storage time and consumption habits, and storage conditions were determined from recordings of temperatures of domestic refrigerators (n = 57). A probabilistic model was designed for the evaluation of the growth of *L. monocytogenes* at each stage of the product pathway from production to consumption, using Monte Carlo simulations and employing the @Risk software. Risks associated to consumption of vacuum-packed and sliced-at-retail head cheese were assessed: The model predicted that the risk of listeriosis per serving of vacuum-packed product was in the 10^{-4} and 10^{-6} range (mean) for the high-risk and general populations respectively, and listeriosis cases were estimated to be greater than those due to consumption of sliced product (with risks in the range of 10^{-7} and 10^{-8}). Overall, the model predicted that the mean number of listeriosis cases ranged from 0.001 to 0.24 and from 0.06 to 10 per one hundred thousand people, for the healthy and the high-risk population, respectively. Scenario analyses indicated that better control of the temperature of domestic refrigerators is effective in reducing the predicted risk of listeriosis for the longer stored vacuum-packed product by ~80 % for both the healthy and high-risk populations, whereas a shorter use-by-date of 30 days is an effective risk mitigation measure for both types of packed product. Model assumptions, as well as data gaps are discussed.

1. Introduction

Listeria monocytogenes is widely distributed in the environment and has the ability to survive and grow in harsh conditions, such as refrigeration temperatures, a wide pH range and high salt concentration (Matle et al., 2020). The bacterium has been isolated from a variety of biotic and abiotic sources, including foods with the oral route being the main mechanism of entry for both animals and humans (Orsi et al., 2011). Listeriosis is a relatively uncommon foodborne illness in the EU, presenting 0.42 cases per 100,000 population according to the ECDC (European Centre for Disease Prevention and Control (ECDC), 2020). Nevertheless it can be very serious for high-risk populations including pregnant women, the elderly (over 65) and individuals with compromised immune systems (Buchanan et al., 2017). Symptoms may present in the gastrointestinal tract alone, but the bacterium may also invade other parts of the body, potentially causing septicemia, meningitis,

Abbreviations: RTE, Ready to Eat; QMRA, Quantitative Microbiological Risk Assessment.

* Correspondence to: C. Hadjicharalambous, Department of Chemistry, University of Crete, Voutes University Campus, Vasilika Vouton, Heraklion 70013, Greece.
** Corresponding author.
E-mail addresses: chad@uoc.gr (C. Hadjicharalambous), beniamino.cencigoga@unipg.it (B. Cenci-Goga).

https://doi.org/10.1016/j.ijfoodmicro.2022.109843
Received 26 November 2021; Received in revised form 23 June 2022; Accepted 17 July 2022
Available online 29 July 2022

encephalitis, spontaneous abortion and stillbirth (U.S. Food and Drug Administrator (FDA)/U.S. Department of Agriculture (USDA), 2003). Reportedly, most invasive listeriosis cases appear as sporadic infections that are often not recorded and the number of detected outbreaks is usually low (EFSA (European Food Safety Authority), 2018). The EU has seen an increase in listeriosis cases in the recent years; EFSA reported that the number of confirmed human invasive listeriosis cases in the EU was 60 % higher in 2015 (2206 cases) than in 2008 (1381 cases) and the vast majority of cases (98 %) appeared as sporadic infections and of domestic origin (28 %). In its annual epidemiological report for 2017, ECDC reported 2502 confirmed listeriosis cases (European Centre for Disease Prevention and Control (ECDC), 2020).

Because of its survival under extreme conditions and its ability to colonise food-processing equipment and surfaces in the form of biofilms, *L. monocytogenes* has inevitably become a great concern for the food industry as traditional cleaning and disinfecting procedures may be inadequate (Gray et al., 2018). Possible entry routes of the bacterium to the final product are both the raw material contamination and cross-contamination during food processing.

Several studies have shown RTE foods to be one of the most important vehicles responsible for human infections (Kurpas et al., 2018). RTE foods typically associated with human listeriosis, include “meat and meat products,” “fish and fish products,” and “milk and milk products”, food of plant origin as well as frozen foods. In the 2018 EFSA scientific opinion on listeriosis (EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2018), which considers foods of animal origin, it was reported that cooked meat and heat-treated sausages were the RTE food subcategories with most consumed servings per person and per year in the EU/EEA. Simultaneously, cooked meat products were associated with the highest number of listeriosis cases per year estimated to be >850. Depending on the formulation and storage conditions, almost all RTE foods may support the growth of *L. monocytogenes* and therefore have the potential to cause disease, especially when consumed by people considered to be at higher risk, such as the elderly, pregnant women and those with pre-existing illnesses which compromise the effectiveness of their immune system.

The food safety criteria laid out in the European Union (EU) Regulation 2073/2005 (Anonymous, 2005) and in its 1441/2007 modification (Anonymous, 2007) specify that: for RTE foods able to support the growth of *Listeria monocytogenes*, there is a requirement for "not detected" in 25 g (with a sampling plan of n = 5) “before the food has left the immediate control of the food business operator, who has produced it”, but allow for up to 100 CFU/g for “products placed on the market during their shelf-life”. The requirement for “not detected in 25g”, applies when the food business operator is not able to demonstrate that the pathogen in the product will remain below the limit of 100 CFU/g throughout its shelf life. The same 100 CFU/g limit also applies throughout the shelf-life of RTE foods that do not support the growth of *L. monocytogenes*.

Prevalence and contamination data on various RTE food categories (meat, milk, fish and their products) marketed in the EU were considered in the 2018 EFSA opinion, against the public health risk they pose when contaminated with *L. monocytogenes*. However, data on the Italian head cooked meat product are not presented in this opinion.

Up to date, neither contamination levels nor a risk assessment study for the Italian Head Cheese are available in literature. The traditionally cooked deli meat product, named “Coppa di Testa”, is produced seasonally by several small and large processing establishments in Italy, using local pork (or hog) meat. Different variations of the head cheese are also manufactured in several parts of the world, including the EU and the USA and have been previously linked to outbreaks of invasive listeriosis (Centers for Disease Control and Prevention (CDC), 2011; Duranti et al., 2018). Coppa di Testa has been identified as a product that can support the growth of *L. monocytogenes* once contaminated with the bacterium, therefore posing a potential risk for public health (Bardasi et al., 2010).

The objective of this study was to estimate the public health risk from *L. monocytogenes* following consumption of the head cheese product, by applying a QMRA model, thus generating useful information for the risk managers. As a scientific process, risk assessment determines the relationship between exposure to a given hazard under a defined set of conditions and the likelihood of an adverse health effect or disease (Koutsoumanis and Aspridou, 2016; McLauchlin et al., 2004). The methodology and the steps applied for the QMRA are described below. The resulting risks and listeriosis cases for a vacuum-packed and a sliced-at-retail product were estimated separately. At the same time, two mitigation strategies were evaluated: shortening the shelf-life at the retail and improving the domestic storage temperature. Results and potential risk management options are discussed in the context of model assumptions and data gaps.

2. Materials and methods

2.1. Head cheese production process, composition and pathway

The production of the Italian head cheese Coppa di Testa was recorded following visits to different production facilities in the area of Umbria, Italy. The traditional cooked pork salami is produced by deboned head meat with the addition of tongue and rind. Generally, the process involves mixing the raw materials and cooking at high temperature (100 °C) for 3 h. The head meat may be deboned before or after cooking, depending on the production method. Salt (2.5 %) and spices (pepper, nutmeg, orange peel, cinnamon) are added to the cooked meat and mixed. The product is placed in molds and allowed to cool overnight, before being portioned, vacuum-packaged in portions of 2–3 kg, and refrigerated. The product is then transported to retail stores, where it can be sold sliced or vacuum- packed in smaller portions (200–500 g), which are often prepared and sealed at the retail store. According to the industry, the product is distributed to the retail shops within 1–2 days from production (expert advice).

2.2. 2.2. Prevalence and concentration data

Data describing the frequency of contamination with *L. monocytogenes* for the Italian head cheese at industry level are not available in literature and were therefore obtained from the Italian Health Ministry[1]: i) sample analysis (n = 528, samples collected during 2018–19) and ii) sample analysis (n = 1040, samples collected between 2016 and 18).

Analysis of the samples above was carried out following the methodology given by the ISO standards 11290-1 and 11290-2 (horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp.). Briefly, 25 g of sample were added to 225 ml of half-Fraser broth (Oxoid, Basingstoke, UK) to obtain a tenfold dilution and then homogenized using a stomacher (PBI International, Milan, Italy). For the enumeration method, 0.1 ml of the initial suspension and of further dilutions was distributed by means of sterile pipette on petri dishes containing Agar *Listeria* Ottaviani and Agosti (ALOA, Oxoid). To estimate low numbers of *L. monocytogenes*, the limit of detection was raised by a factor of 10 by inoculating 1 ml of the initial suspension on three petri dishes. The inoculum was spread over the surface of the agar plates with a sterile spatula. Plates were incubated at 37 °C for 24 h and for additional 24 h if no growth was observed. Blue-green colonies surrounded by an opaque halo were presumptively identified as *Listeria* spp., isolated and stored at −80 °C for the identification. For the detection method, the primary enrichment medium was incubated at 30 °C for 24 h. After the incubation, 0.1 ml was transferred to a tube containing 10 ml of Fraser broth (Oxoid). Tubes were incubated at 37 °C

[1] Italian Health Ministry, Piano di controllo nazionale pluriennale, 2020 (https://www.salute.gov.it/pianoNazionaleIntegrato2020/homePianoNazionaleIntegrato2020.jsp).

for 24 h. Both from the primary enrichment culture and from the secondary, samples were inoculated by means of a sterile loop on the surface of petri dishes of ALOA (Oxoid). Plates were incubated at 37 °C for 24 h and for additional 24 h if no growth was observed. Blue-green colonies surrounded by an opaque halo were presumptively identified as *Listeria* spp., isolated and stored at −80 °C for the identification. *Listeria* spp. colonies were identified by the API *Listeria* kit (BioMerieux, Marcy l'Etoile, France).

2.3. Temperature conditions in domestic refrigerators in Italy

Temperature data were collected from 57 domestic refrigerators in the area of Perugia, Italy, using electronic temperature-monitoring data loggers (Testostor 175-2, Testo, Lenzkirch, Germany). The 57 domestic refrigerators is a sample of the typical customer of RTE and the customers were selected with a questionnaire at several shops. The data loggers were placed at the middle of the door shelves of the refrigerators and were programmed to record the air temperature every 5 min for 7 days. The data were analysed and used to describe the domestic storage temperature of the product.

2.4. Consumer survey

Information on Coppa di Testa domestic storage and consumption habits of consumers was derived from a consumer survey. A relevant questionnaire was prepared and distributed via web-based social network platforms and in printed form as we previously described in (Hadjicharalambous et al., 2019). Briefly, out of 162 responders, 39 % were men and 61 % were women. Among these, 20 % of men and 10 % of women were aged 65 or older. Information (n = 162) was collected on the size (portions) and frequency (per week) of head cheese consumption as well as the time required for product transport to home and the time of domestic storage before consumption for both vacuum-packed and sliced at retail product. The data were analysed and used as inputs in the model as described in Risk characterization modules.

2.5. Risk assessment - model overview

The most common route through which *L. monocytogenes* can contaminate RTE cooked meat products is during post-process handling (i.e. after the cooking step) (Mataragas et al., 2010; U.S. Food and Drug Administrator (FDA)/U.S. Department of Agriculture (USDA), 2003). The intrinsic factors of head cheese do not prevent the growth of *Listeria* and additionally, the product has a rather long shelf life (up to 180 days) during which it can be consumed. Therefore, post-process contamination with *L. monocytogenes*, even with low cell numbers, may lead to high numbers of the microorganism at the time of consumption, because of its ability to grow at common refrigeration temperatures (Mataragas et al., 2010). Accordingly, in the present study, the quantitative risk assessment was performed with emphasis on the exposure assessment and risk characterization stages after the manufacturing and distribution of the product and up to the time of consumption, i.e. throughout retail, consumer handling and storage before consumption. Overall the risk assessment procedure was based on four separate stages (in accordance with Codex Alimentarius), namely i) hazard identification, ii) hazard characterization, iii) exposure assessment and iv) risk characterization, as we described in (Hadjicharalambous et al., 2019).

In order to perform a quantitative risk assessment, we developed a probabilistic model that implements Monte Carlo simulations using @Risk simulation software (@Risk 8.2 for Excel, Palisade, Ithaca, USA), as an add-in to Microsoft Excel. The mathematical model describes the possibility and levels of post-production contamination with *L. monocytogenes* and the growth of the pathogen, taking into account the effects of temperatures and times, during transport and storage, on the growth of the bacterium. It then calculates the likelihood of public health adverse effects following consumption of the RTE product. Its structure is based on prevalence and concentration data, information derived from the consumer survey, refrigerator survey, previous risk assessments and on consultation with experts. The product pathway and risk assessment process is diagrammed in Fig. 1 below. The mathematical model begins at industry level, as the samples were collected at that point in the pathway.

2.5.1. Hazard identification

Among the 17 distinct species of *Listeria* so far identified (Orsi and Wiedmann, 2016), *Listeria monocytogenes* is the most critical species from a human health perspective, with serious manifestations (meningitis, septicaemia, abortion) and 20–30 % fatality. Full descriptions of the hazard *L. monocytogenes* presents are available in (Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO), 2004) as well as (EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2018).

The EU has seen an increase in listeriosis cases (both outbreaks and sporadic cases) in the recent years (European Centre for Disease Prevention and Control (ECDC), 2018) (EFSA (European Food Safety Authority), 2018).

In addition, details of major food-borne listeriosis outbreaks around the world due to meat products have been reported in recent reviews (Buchanan et al., 2017; Matle et al., 2020). Relevant to this risk assessment, an outbreak of listeriosis with 24 confirmed hospitalizations, in which hog head cheese (Coppa di Testa) was implicated, was reported to have occurred between May 2015 and March 2016 in Italy. The contamination was determined to have originated from a local production plant (Duranti et al., 2018).

2.5.2. Hazard characterization

Within the QMRA, hazard characterization is the stage that includes a 'dose-response model': this describes the fraction of a population who would become ill from consuming a particular number of cells of *L. monocytogenes* (Lammerding and Fazil, 2000). Thus, the consumption frequency and doses of the pathogen can be converted into an estimate for public risk of illness. Such models are difficult to build as the required data are obtained from disease outbreaks, human volunteer feeding trials or animal experiment data and are usually insufficient (Ross et al., 2009).

Several QMRA studies (Bassett et al., 2012; Giovannini et al., 2007; Mataragas et al., 2010; Ross et al., 2009) have applied the FAO/WHO (2004) exponential dose-response model for *L. monocytogenes* (Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO), 2004). The general form of the exponential dose–response model by FAO/WHO was used in this QMRA and is described by the equation:

$$P_{ill} = 1 - e^{(-r.D)}$$

where:

$\mathbf{P_{ill}}$ is the probability of severe illness;

D is the number of *L. monocytogenes* cells consumed per serving (the dose) and is calculated as $\mathbf{D = C \cdot S}$ where **C** is the concentration of the pathogen (number of cells/g) and **S** is the serving size consumed during a meal (g);

r is the parameter that expresses the probability of illness after the consumption of a single *L. monocytogenes* cell for the population group being considered. Median r-values previously generated by FAO/WHO (2004) for 'healthy' ($r = 2.37 \times 10^{-14}$) and 'vulnerable' ($r = 1.06 \times 10^{-12}$) populations, were used in the risk characterization (see section below). The expected incidence of listeriosis per serving consumed was calculated for the two risk sub-populations, based on the distribution of exposure levels.

When applying the above response model equation, the following assumptions are made (Giovannini et al., 2007):

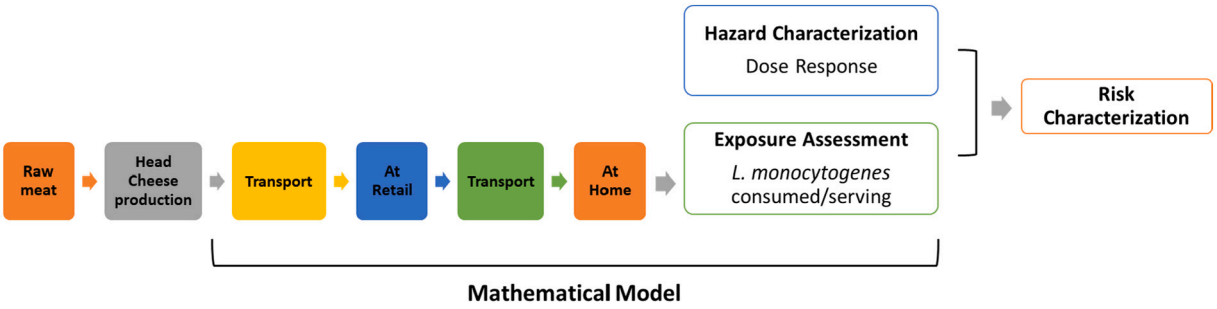

Fig. 1. The product pathway and risk assessment process (modified from (Hadjicharalambous et al., 2019)).

- The exponential model is a non-threshold model, therefore no 'minimum infectious dose' exists.
- At low doses, the model presents a log-linearity (dose (log CFU) vs. log probability of illness); this implies that at low doses, a single serving with a specified level of contamination has the same public health impact as 10 servings with 10-fold fewer organisms.
- The fraction of the total population being at risk i.e. 'susceptible or high-risk' is considered at the 20 % of the total population as previously described (Buchanan et al., 1997; Mataragas et al., 2010).

2.5.3. Exposure assessment

The exposure assessment describes how often and at what levels, consumers in the population consume the hazard in the food of interest. In this risk assessment, the *"number of L. monocytogenes per serving of contaminated Coppa di Testa"* is the important output from the exposure assessment (Food and Drug Administration (FDA)/Health Canada—Santé Canada, 2015). This output is extracted using information about the *frequency of contamination* (prevalence data) and the predicted *final contamination levels at the point of consumption*. The final contamination levels were calculated from initial contamination levels (at the point of industry) followed by an estimation for growth of *L. monocytogenes* based on times and temperatures of distribution and storage prior to consumption, using predictive microbiology. Information on the number of servings consumed and the serving size were used to estimate the dose of the pathogen, and were taken from our consumer survey. A schematic overview of the influence diagram for the exposure assessment is presented in Fig. 2.

2.5.3.1. Prevalence and concentration data. Details of the prevalence and concentration data of the samples are presented in Table 1. Regarding product safety, unsafe products are considered those purchased and consumed within the specified shelf-life, yet contain a *L. monocytogenes* population above the microbiological criterion of 100 CFU/g (Anonymous, 2005; Anonymous, 2007). Certainly, products consumed after expiration could also be unsafe, however this is beyond producers' responsibility. For products consumed within their shelf-life,

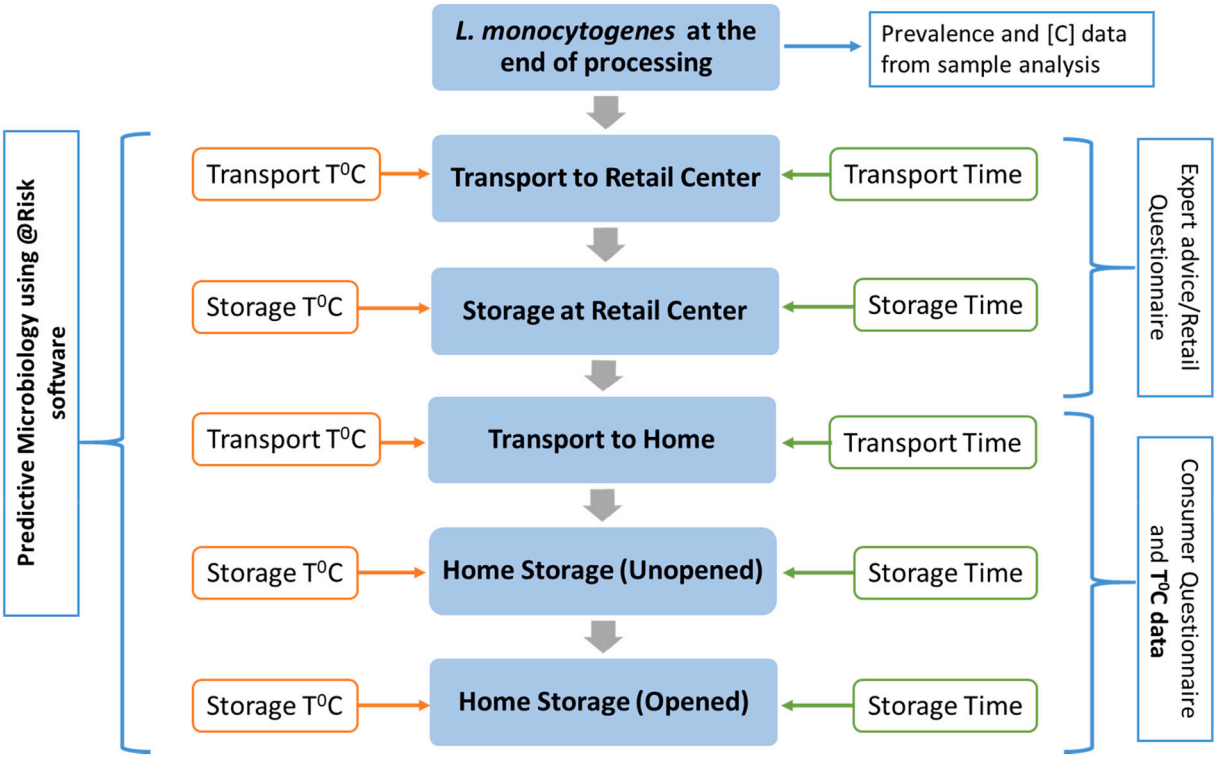

Fig. 2. Influence diagram outlining the overall structure of the exposure assessment part of the model. The various stages between industry, retail and consumption are discretely modelled and the output of each stage is influenced by model inputs as depicted by the arrow points. For each of the stages shown, the numbers of *L. monocytogenes* are calculated, based on the levels at the end of the previous stage and the additional model inputs. Estimated concentrations of *L. monocytogenes* at the time of consumption (final output of the diagram) are then combined with the frequencies (prevalence) and the FAO/WHO (2004) dose–response model to estimate consumer risk. Figure is modified from (Hadjicharalambous et al., 2019).

Table 1
L. monocytogenes concentration data used in the model.

Source of data	n	Prevalence (%)	0.04–40 CFU/g (%)	>100 CFU/g (%)
Italian Health Ministry				
Year 2018[a]	355	8.5	1.1	1.1
Year 2019	173	5.8	1.7	0.6
Italian Health Ministry				
Year 2016	350	4.9	2.3	1.1
Year 2017	337	4.5	1.8	1.8
Year 2018[a]	353	5.7	0.6	0.6
Total or (average)	1568	(5.9)	(1.5)	(1.1)

[a] Data received at different periods.

the above criterion applies only if the producer is able to demonstrate that the maximum number of 100 CFU/g is not exceeded throughout the shelf-life of the product. In the opposite event, the criterion of "not detected in 25g sample" (five sample analysis) must be confirmed for product compliance.

L. monocytogenes concentration data from all years, were combined (n = 1568). The concentrations of *L. monocytogenes* in positive samples ranged from <0.04 CFU/g (presence in 25 g) to >100 CFU/g and were reported at certain discrete values in that range (0.04, 40 or 100 CFU/g). For the modelling, the positive samples were assumed to have a minimum concentration of 0.04 CFU/g and a maximum concentration of 100 CFU/g, which was set as the upper limit of concentration for samples below the microbiological safety criterion. In fact, the actual data in Table 1 show that about 1.1 % of all samples are above the microbiological limit of 100 CFU/g. Therefore, setting this as the upper limit in the model, inevitably leads to the risk being underestimated. More precise reporting of pathogen concentration values is necessary for an accurate estimation of risk.

2.5.3.2. Estimating contamination levels at consumption. There are no data which describe the levels of head cheese contamination with *L. monocytogenes* at the point of consumption. The levels can vary and are influenced by different post-processing (after cooking) factors, most importantly the time and temperature, as shown in Fig. 2. Final contamination levels were estimated using predictive microbiology based on information about: i) known (measured) contamination frequencies and levels after production; ii) times and temperatures from industry to retail and until consumption. The growth of *L. monocytogenes* was considered only in vacuum-packed or open-sliced products. Modified atmosphere-packed products were not considered.

2.5.4. Risk characterization

The risk assessment model is composed of four modules: (a) industry and transport to retail, (b) retail, (c) transport to home, and (d) consumption, dose-response and risk of infection, which calculate the estimated exposure to *L. monocytogenes* (pathogen concentration per serving) and use a dose-response function to predict the risk of listeriosis. The model was built in Excel spreadsheet (Microsoft, version 2016) and the simulations were carried out using @Risk software version 8.2 (Palisade Corporation). A total of 100,000 iterations using Monte Carlo sampling were run in one simulation for each scenario. Applying the simulations at 1,000,000 iterations gave equal results. The random generator seed was fixed at 1 to guarantee that results were repeatable and different scenarios could be compared. The final outputs of this QMRA model, both for the general and high-risk populations, were *the risk of listeriosis per serving* (probability of infection due to consumption of one serving) and *the number of listeriosis cases in a population of 100,000*.

2.5.4.1. Modules. The parameters and procedures for each module are shown in Tables 2-5. Table 2 lists the steps of the "industry & transport to retail module". Starting from prevalence results from the analysis of 1568 samples, the initial *L. monocytogenes* concentration in contaminated samples is described by a Cumulative distribution considering a minimum and a maximum pathogen concentration of −1.4 and 2 log CFU/g, respectively. This is used to calculate the initial concentration in any given product sample through a Discrete distribution that combines the prevalence and concentration data of both positive and negative samples. It was assumed that post-contamination at production was the only source of pathogen and changes in its prevalence or concentration due to cross-contamination during distribution, handling (i.e. slicing at the retail) and storage (retail and home) were negligible. Times and refrigeration temperatures during storage at production facility and distribution to retail were obtained from literature or after discussions with experts from the Italian RTE meat industry. Values of mean, most likely and maximum were modelled using the Pert distribution.

The growth of *L. monocytogenes* is described by the relationship between growth rate and temperature represented by a generalized linear regression model shown in Eq. (1) (Ratkowsky et al., 1982):

$$r = b\,(T - T_0) \tag{1}$$

where: r is the square root of maximum growth rate (μ), b is the slope of the regression line, T is the temperature (°C) and T_0 is the theoretical minimum temperature for microbial growth. Kinetic parameters specifically for the growth of *L. monocytogenes* according to the square root model in a vacuum-packed sliced cooked meat product, were determined, as shown in Eq. (2), which concerns the growth rate per day (i.e. logCFU/day) (Mataragas et al., 2006):

$$\sqrt{\mu} = 0.058(T + 1.03) \tag{2}$$

Similarly, Szczawiński et al. (2017) determined the parameters of *L. monocytogenes* growth in an open cooked ham product to be well described by the polynomial Eq. (3), which calculates the growth rate per hour (i.e. logCFU/h):

$$\mu = 0.000893 - 0.00^{*}T + 0.0000439^{*}T^2 \tag{3}$$

Eqs. (2) and (3) were used to calculate *L. monocytogenes* growth during transportation and storage at the various stages described in the modules, either for the vacuum-packed or open (sliced at retail) product. Pathogen concentration at each stage was calculated as the sum of its initial (or previous) concentration and the subsequent growth during the refrigerated shelf-life.

The retail module is described in Table 3. The distribution of temperatures during retail storage was retrieved from Koutsoumanis et al. (2010), who contacted a study for dairy product display cabinets in Greece and reported a mean of 4.98 °C and a standard deviation of 2.90 °C. Similarly, Gogou et al. (Gogou et al., 2015) reported an average temperature of 4.0 °C during display of meat products at retail in Greece, in agreement with a comparable study in France (Mercier et al., 2017). Expert advice from industry (four Italian production facilities) as well as product packaging/labelling information was used to derive shelf lives for Coppa di Testa, which ranged from 1 to 6 months i.e., some labels also included the date of manufacture from which the nominal shelf life of the product, specified by the manufacturer, could be determined. Six months was used as the maximum time of storage at the retail and it was assumed that 95 % of the product is consumed within 45 days of production similar to previous literature reports on RTE meat products (Mataragas et al., 2010) and that only 5 % remains available after this period i.e. for the remaining 135 days. Storage time was described by the Uniform distribution.

Table 4 shows the parameters of the module concerning growth of the pathogen during transport to home and before domestic storage. One important aspect of transport to home is the potential for significant temperature change as the product is transported under non-refrigerated conditions. To calculate the average product temperature during

Table 2
Summary of variables for "Industry & Transport to Retail Module".

Parameter description	Notation	Units	Value	Source
Prevalence of *L. monocytogenes* positive samples after production (positive samples)	$P_{positive}$	%	=RiskBeta(92,1568) Beta(a1,a2), where a1 = s + 1 and a2 = n-s + 1	Sample analysis
Prevalence of samples with *L. monocytogenes* below detection limit (negative samples)	$P_{negative}$	%	=1-$P_{positive}$	Calculated
L. monocytogenes concentration in positive samples	$LMC_{positive}$	log CFU/g	=RiskCumul(−1.4,2, (−1.4,1.6,2}, (0.2,0.3,1})	(Mataragas et al., 2010) Assumption: the max concentration in positive samples is 100 CFU/g
L. monocytogenes concentration in negative samples	$LMC_{negative}$	log CFU/g	=RiskUniform(−4,−1.4)	(U.S. Food and Drug Administrator (FDA)/U.S. Department of Agriculture (USDA), 2003) and (Mataragas et al., 2010). Min and max values give a mean of −2.70 logCFU/g.
Initial *L. monocytogenes* concentration	$LMC_{initial}$	log CFU/g	=RiskDiscrete($LMC_{positive}$: $LMC_{negative}$,$P_{positive}$:$P_{negative}$)	Calculated
** For the following steps we consider that the product is stored at the industry and then distributed to the retail under vacuum-packed conditions - uncertainty of sampling time for microbiological analysis*				
L. monocytogenes growth rate as a function of temperature (vacuumed-packed)	$LM\mu_{growth}$	log CFU/day	= $(0.058*T + 0.058*1.03)^2$	(Mataragas et al., 2006)
Storage time at industry	$ST_{industry}$	days	=RiskPert(0.1,1,7)	Uncertainty of storage time at industry. Modelling of experts' opinion.
Temperature during storage at industry	$T_{industry}$	^{0}C	=RiskPert(2,4,6)	Uncertainty of storage temperature at industry. Modelling of experts' opinion.
L. monocytogenes growth rate as a function of temperature at industry	$LM\mu_{industry}$	log CFU/day	$=(0.058*T_{industry} + 0.058*1.03)^2$	Calculated
L. monocytogenes concentration in product after storage at industry	$LMC_{industry}$	log CFU/g	$=LMC_{initial} + LM\mu_{industry} * ST_{industry}$	Calculated
Transport time during distribution	$ST_{distribution}$	days	=RiskPert(0.05, 0.15, 0.5)	(Mataragas et al., 2010)
Temperature during distribution	$T_{distribution}$	^{0}C	=RiskPert(5,7,12)	(Mataragas et al., 2010)
L. monocytogenes growth rate as a function of temperature during distribution	$LM\mu_{distribution}$	log CFU/day	$=(0.058*T_{distribution} + 0.058*1.03)^2$	Calculated
L. monocytogenes concentration after distribution	$LMC_{distribution}$	log CFU/g	$=LMC_{industry} + LM\mu_{distribution} * ST_{distribution}$	Calculated

transport we used data from FDA/USDA (U.S. Food and Drug Administrator (FDA)/U.S. Department of Agriculture (USDA), 2003) and modelling steps previously reported by Mataragas et al. (Mataragas et al., 2010). Minimum and maximum times for transport to home were derived from our consumer survey and fitted in a Uniform Distribution. *L. monocytogenes* growth as a function of temperatures and times during transport to home was modelled separately for vacuum-packed or open/sliced product, assuming that the consumer may opt for the first or second scenario when purchasing the product at the retail.

The description of consumption, dose-response and risk of infection module is shown in Table 5. Domestic storage temperature data were obtained from the refrigerator survey and described in a Normal Distribution as N(8.81;2.91) °C. Domestic storage times were derived from the consumer survey. Based on the responses, a sliced at retail RTE meat product is most likely to be consumed within 1.7 (minimum) to 3.4 (maximum) days of purchase, whereas a vacuum-packed product is more likely to be stored between 7.4 (minimum) and 11.3 (maximum) days before opening. Consequently, it was assumed that after opening, vacuum-packed products are most likely to be consumed within 1.7–3.4 days. These data were described as minimum and maximum values in Uniform Distributions. For calculating *L. monocytogenes* concentration after domestic storage in either vacuum-packed or open/sliced product, the maximum population density was set to 8.5 log CFU/g.

Final *L. monocytogenes* concentration in a serving of Coppa di Testa was calculated from serving size and final pathogen concentration at the end of the product pathway, using a Poisson distribution (Condoleo et al., 2017), as described in Hazard Characterization. The serving size was estimated based on the weight of a slice (own measurements, Normal Distribution) and the number of slices served per meal retrieved from the consumer survey.

Data from fifty-seven responders who consume Coppa di Testa (out of 162 responders in the consumer survey) were analysed. According to consumer responses, 0.5, 0.5 and 23 slices are consumed within a week and these were set as the minimum, most likely and maximum values, respectively, of a Triangular distribution. This is then divided by 21 to calculate the number of slices consumed per meal.

The dose-response function described in Hazard Characterization with discrete "r" parameters for general (2.37×10^{-14}) and vulnerable (1.06×10^{-12}) populations was used to estimate the risk of invasive listeriosis from bacteria consumed from a single serving (Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO), 2004). The number of listeriosis cases in a population of 100,000 for both the general and high-risk population was then estimated using a binomial distribution (Campagnollo et al., 2018). The outputs of two alternative scenarios proposed as potential interventions were evaluated.

3. Results & discussion

3.1. Temperature conditions in domestic refrigerators in Italy

Domestic storage temperatures were estimated by collecting and analysing temperature data of 57 domestic refrigerators in the area of Perugia, Italy (Cenci-Goga et al., 2002). The distribution of mean temperature and the temperature profiles of the refrigerators are shown in Fig. 3 and Table 6.

Data analysis suggested that temperatures for the majority of domestic refrigerators were in the range of 4–14 °C with a mean temperature of 8.8 °C (SD 2.92 °C). Nevertheless, 26 % of the refrigerators showed temperature fluctuations above 14 °C, even for a small percentage of the recording time. Previous European surveys in Greece (Tsaloumi et al., 2021) and Spain (Jofré et al., 2019) reported lower average values of 5.97 °C (SD 2.73 °C) and 5.4 °C (SD 2.3 °C) respectively, with 40–50 % of the domestic refrigerators operating at temperatures >6 °C. However, an analysis of twelve studies by Roccato et al. (Roccato et al., 2017) has shown that the overall variability of European

Table 3
Summary of variables for "Retail Module".

Parameter description	Notation	Units	Value	Source
L. monocytogenes concentration in product after distribution – *Input*	$LMC_{distribution}$	log CFU/g	$=LMC_{industry} + LM\mu_{distribution} * ST_{distribution}$	From the Industry module
Temperature during storage at retail	T_{retail}	^0C	=RiskNormal (4.98, 2.9)	(Koutsoumanis et al., 2010)
Storage time at retail	$ST_{retail-1}$	days	=RiskUniform (0, 45)	(Bassett et al., 2012; Mataragas et al., 2010)
	$ST_{retail-2}$	days	=RiskUniform (0, 135)	Assumption: 5 % of the product is sold between day 46–180
L. monocytogenes growth rate as a function of temperature at retail	$LM\mu_{retail}$	log CFU/day	$=(0.058*T_{retail} + 0.058*1.03)^2$	Calculated
L. monocytogenes concentration after retail storage – (0–45 days)	$LMC_{retail-1}$	log CFU/g	$=LMC_{distribution} + LM\mu_{retail} * ST_{retail-1}$	Calculated
L. monocytogenes concentration after retail storage – (46–180 days)	$LMC_{retail-2}$	log CFU/g	$= LMC_{retail-1} + LM\mu_{retail} * ST_{retail-2}$	Calculated[a]
L. monocytogenes concentration after retail storage	LMC_{retail}	log CFU/g	=RiskDiscrete ($LMC_{retail-1}$: $LMC_{retail-2}$,0.95:0.05)	Calculated

[a] Iterations that resulted in concentration outputs above 8.5 log CFU/g were not considered.

domestic refrigerators is represented by a mean temperature of 7.0 °C (SD 2.7 °C) for southern countries, and, 6.1 °C (SD 2.8 °C) for the northern countries, values that approximate our data. It becomes thus evident that the under-performance of European domestic refrigerators, which operate with mean temperatures above the ideal 4 °C, is especially critical for assuring product safety and quality, as it concerns the last step in the cold chain.

3.2. Risk assessment

3.2.1. Baseline scenario outputs

The growth of *L. monocytogenes* in Coppa di Testa meat products from production to home was predicted using a QMRA model based on the prevalence and initial concentration of the pathogen and the storage temperature and time during its shelf-life. The maximum population density for *L. monocytogenes* was set to 8.5 log CFU/g according with the maximum population density used in previous risk assessments and the experimentally observed growth on similar RTE products (Szczawiński et al., 2017; Tsaloumi et al., 2021).

Simulation of the QMRA model resulted in estimations of *L. monocytogenes* levels at the various steps of the pathway. Specifically, the estimated distributions of *L. monocytogenes* levels at the point of industry are shown in Fig. 4 with a mean concentration of -2.5 ± 1.0 log CFU/g, taking into account both contaminated and non-contaminated products based on prevalence data from sample analysis. An important assumption of this QMRA is that cross-contamination during subsequent steps (retail sale and domestic storage) does not occur. Simulation of pathogen growth during transportation to the retail showed that the product reaches the supermarkets with a mean of -2.4 ± 1.0 log CFU/g, therefore growth from industry to retail is negligible. Subsequently, based on temperature and storage time profiles used as inputs in the model, the pathogen grows to a mean of -0.9 ± 1.8 log CFU/g just before transportation to home; at this point 92 % of products have a pathogen concentration below the microbiological safety criterion of 100 CFU/g.

Following, the growth of *L. monocytogenes* in a product purchased as (a) vacuum-packed or (b) open/sliced from the retail was considered

Table 4
Summary of variables for "Transport to Home Module".

Parameter description	Notation	Units	Value	Source
L. monocytogenes concentration after retail storage - *Input*	LMC_{retail}	log CFU/g	=RiskDiscrete($LMC_{retail-1}$: $LMC_{retail-2}$,0.95:0.05)	From the Retail Module
Ambient temperature	$T_{ambient}$	^0C	=RiskPert(0,20,40)	(U.S. Food and Drug Administrator (FDA)/U.S. Department of Agriculture (USDA), 2003)
Max change in temperature during transport to home	ΔT_{max}	^0C	$=T_{ambient} - T_{retail}$	Calculated
Potential change in temperature during transport	$T_{potentialchange}$	^0C	=RiskNormal (3.72, 2.82)	(U.S. Food and Drug Administrator (FDA)/U.S. Department of Agriculture (USDA), 2003)
Change in temperature during transport	T_{change}	^0C	$=IF(\Delta T_{max} \leq 0, 0, T_{potentialchange})$	Calculated
Product temperature after transport	$T_{product}$	^0C	$=T_{retail} + T_{change}$	Calculated
Average product temperature during transport	$T_{product\ average}$	^0C	$=Average(T_{retail}, T_{product})$	Calculated
Transport time from retail to home	$Tt_{transport}$ $Tt_{transport-h}$	Days Hours	=RiskUniform(10.9,30.35)/1440 =RiskUniform(10.9,30.35)/60	Consumer survey (min, max). Transport time in minutes converted to days (1 day = 1440 min) or hours (1 h = 60 min).
(a) Modelling growth for vacuum-packed product				
L. monocytogenes growth rate as a function of temperature during transport	$Lm\mu_{transport-v}$	log CFU/day	$=(0.058*T_{product\ average} + 0.058*1.03)^2$	Calculated
L. monocytogenes concentration after transport to home	$LMC_{transport-v}$	log CFU/g	$=LMC_{retail} + LM\mu_{transport-v} * Tt_{transport}$	Calculated
(b) Modelling growth for open/sliced product				
L. monocytogenes growth rate as a function of temperature during transport	$Lm\mu_{transport-o}$	log CFU/h	μ (logCFU/h) = $0.000893–0.00*T + 0.0000439*T^2$	(Szczawiński et al., 2017)
L. monocytogenes concentration after transport to home	$LMC_{transport-o}$	log CFU/g	$=LMC_{retail} + LM\mu_{transport-o} * Tt_{transport-h}$	Calculated

Table 5
Summary of variables for "Consumption, dose-response and risk of infection module".

Parameter description	Notation	Units	Value	Source
Temperature at domestic storage	^{0}C	$T_{domestic}$	=RiskNormal(8.81,2.91)	Own data from data loggers in 57 refrigerators
(a) Modelling growth for vacuum-packed product				
L. monocytogenes concentration after transport to home – *input*	$LMC_{transport\text{-}v}$	log CFU/g	$=LMC_{retail} + LM\mu_{transport\text{-}v} * Tt_{transport}$	From the "Transport to Home" Module
Domestic storage time before package opening	Days	$ST_{domestic\text{-}V}$	=RiskUniform(7.39,11.32)	Consumer survey
L. monocytogenes growth rate in vacuum-packed product as a function of domestic temperature	log CFU/day	$LM\mu_{domestic\text{-}V}$	$=(0.058*T_{domestic} + 0.058*1.03)^2$	Calculated
L. monocytogenes concentration in vacuum-packed product after domestic storage	log CFU/g	$LMC_{domestic\text{-}V}$	$=LMC_{transport\text{-}V} + Lm\mu_{domestic\text{-}V} * ST_{domestic\text{-}V}$	Calculated; Assumption: shelf life is not exceeded
Domestic storage time after package opening	Days	$ST_{domestic\text{-}O}$	=RiskUniform(1.72,3.44)	Consumer survey
L. monocytogenes growth rate in open product as a function of domestic temperature	log CFU/h	$LM\mu_{domestic\text{-}O}$	μ (logCFU/h) = 0.000893–0.00*T + $0.0000439*T^2$	Calculated
L. monocytogenes concentration after domestic storage	log CFU/g	$LMC_{domestic\text{-}V+O}$	$=LMC_{domestic\text{-}V} + LM\mu_{domestic\text{-}O} * ST_{domestic\text{-}O}*24$	Calculated, multiplied by 24 h/day; Assumption: shelf life is not exceeded; max population density set to 8.5 log CFU/g
(b) Modelling growth for open/sliced product				
L. monocytogenes concentration after transport to home – *input*	$LMC_{transport\text{-}o}$	log CFU/g	$=LMC_{retail} + LM\mu_{transport\text{-}o} * Tt_{transport\text{-}h}$	From the "Transport to Home" Module
Domestic storage time for open/sliced product	Days	$ST_{domestic\text{-}O}$	=RiskUniform(1.72,3.44)	Consumer survey
L. monocytogenes growth rate in open product as a function of domestic temperature	log CFU/h	$LM\mu_{domestic\text{-}O}$	μ (logCFU/h) = 0.000893–0.00*T + $0.0000439*T^2$	Calculated
L. monocytogenes concentration after domestic storage	log CFU/g	$LMC_{domestic\text{-}o}$	$= LMC_{transport\text{-}o} + LM\mu_{domestic\text{-}o} * ST_{domestic\text{-}o}*24$	Calculated, multiplied by 24 h/day Assumption: shelf life is not exceeded; max population density set to 8.5 log CFU/g
Modelling dose at consumption (log CFU/serving)				
Weight of a slice	grams	W_{slice}	=RiskNormal(0.067,0.006)	Measured in product
Number of slices served per meal	number	N_{slice}	=RiskTriang(0.5,0.5,23.096)/21	Consumer survey
Serving size per meal	grams	$S_{serving}$	$=N_{slice} * W_{slice}$	Calculated; Assumption: serving size is the same among the general and high-risk population.
L. monocytogenes concentration in a serving (dose) of vacuum-packed product	log CFU/ serving	$LMC_{serving\text{-}V+O}$	$=$RiskPoisson $(S_{serving}*LMC_{domestic\text{-}V+O})$	(Condoleo et al., 2017)
L. monocytogenes concentration in a serving (dose) of open/sliced product	log CFU/ serving	$LMC_{serving\text{-}O}$	$=$RiskPoisson $(S_{serving}*LMC_{domestic\text{-}O})$	(Condoleo et al., 2017)
Modelling risk				
Parameter "r" for dose response for general population	–	$r_{general}$	2.37×10^{-14}	(Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO), 2004)
Parameter "r" for dose response for high-risk population	–	$r_{high\text{-}risk}$	1.06×10^{-12}	(Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO), 2004)
Risk of listeriosis per serving for general population	–	$Risk_{general}$	$=1\text{-}Exp(-r_{general}*LMC_{serving\text{-}V+O})$	Calculated, (Buchanan et al., 1997)
Risk of listeriosis per serving for high-risk population	–	$Risk_{high\text{-}risk}$	$1\text{-}Exp(-r_{high\text{-}risk}*LMC_{serving\text{-}V+O})$	Calculated, (Buchanan et al., 1997)
Risk of listeriosis per serving for general population	–	$Risk_{general}$	$=1\text{-}Exp(-r_{general}*LMC_{serving\text{-}O})$	Calculated, (Buchanan et al., 1997)
Risk of listeriosis per serving for high-risk population	–	$Risk_{high\text{-}risk}$	$1\text{-}Exp(-r_{high\text{-}risk}*LMC_{serving\text{-}O})$	Calculated, (Buchanan et al., 1997)

separately and modelled after the equations suggested by Mataragas et al. (Mataragas et al., 2006) and Szczawiński et al.Szczawiński et al. (2017) respectively, yielding different final concentration outputs. Consequently, final concentrations at the point of consumption range from −3.6 to 8.5 log CFU/g with a mean of 2.5 ± 2.4 log CFU/g for the vacuum-packed, and from −3.7 to 8.2 log CFU/g with a mean of −0.66 ± 1.7 log CFU/g for the open/sliced product.

This difference in mean values is not surprising, and could be related to the longer storage time for the vacuum-packed product after purchase, and the fact that the presence of oxygen does not affect the pathogen's growth during this time. The growth of lactic acid bacteria (LAB) which act as nutrient competitors, the addition of NaCl as food preservative and the possible addition of $NaNO_2$ as chemical preservative, are additional factors that affect the growth of *L. monocytogenes* in RTE meat products (Mejlholm and Dalgaard, 2015; Tsaloumi et al., 2021) but are not considered in this model, as relevant data were not available. It should be noted that the temperature is considered as the most important factor influencing *L. monocytogenes* growth in meat products, since the concentrations of sodium nitrites usually found in cooked, cured meat products do not prohibit its growth (Mataragas et al., 2006). In addition, as a traditional product, methods for production of Coppa di Testa do not always include the addition of nitrites (expert advice). Therefore, given this approximation, the two pathways of the model differentiate and essentially reflect the effect of the length of refrigerated storage time and control of temperature at the consumer level to the final growth of the pathogen, as previously reported (Tsaloumi et al., 2021). The probability distributions for the final concentrations at the point of consumption are shown in Fig. 5.

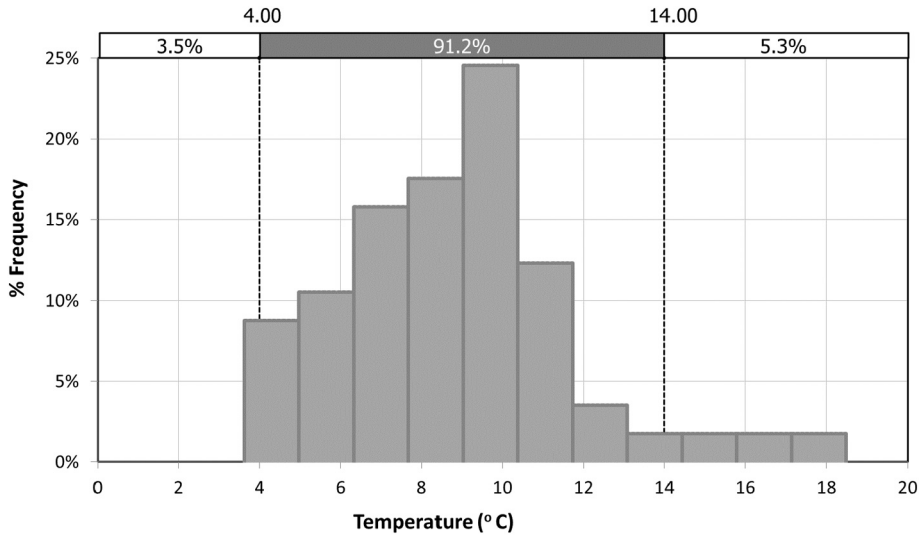

Fig. 3. Distribution of mean temperature in 57 domestic refrigerators tested in Perugia, Italy.

Table 6
Temperature data for domestic refrigerators (n = 57).

Statistical parameter	Mean	Std Dev	Minimum	Maximum
Value (temperature, °C)	8.80	2.91	3.62	18.49

Temperature fluctuations						
Temperature (°C)	>4	>6	>8	>10	>12	>14
% refrigerators	100	98	93	75	53	26
% time	96	84	57	28	10	5

The main outputs of the QMRA model, regarding the risk of listeriosis are presented in Table 7. It is important to note that the simulation only considers the effects of temperature and time on pathogen concentration during retail and domestic storage as well as transportation. As expected, high-risk populations such as the elderly >65 and immunocompromised, display a higher risk of listeriosis compared to the general population. We predicted that consumption of any given sample of Coppa di Testa would cause a mean of 10 cases of listeriosis in a vulnerable population of 100,000 if the product is purchased as vacuum-packed, stored in a domestic refrigerator before opening and consumed, according to recorded consumer habits. If the product is purchased as open/sliced at the retail, its consumption would cause a mean of 0.06 cases of listeriosis in a high-risk population of 100,000, providing an indication that lower storage time at consumer level could reduce risk.

According to the ECDC annual epidemiological report on listeriosis, the Italian case rate was 0.3 per 100,000 population for 2010 and 0.2 per 100,000 population for the following three years (European Centre for Disease Prevention and Control (ECDC), 2020). From the results of the QMRA presented in Table 7 (mean values and SD), it is evident that the contribution of Coppa di Testa to the total national case rate is predicted to arise mainly from the consumption of the longer stored vacuum packed product by people in the high-risk group. This category also yields the highest maxima in the "case rate per 100,000" and the "risk per serving". These "tails" in the model outputs result from simulations that combine high doses at consumption (from high initial contamination level (Fig. 4) and a large serving size) with a high "r" parameter for the vulnerable populations. In addition, the longer storage time for the vacuum-packed product increases the probability for higher

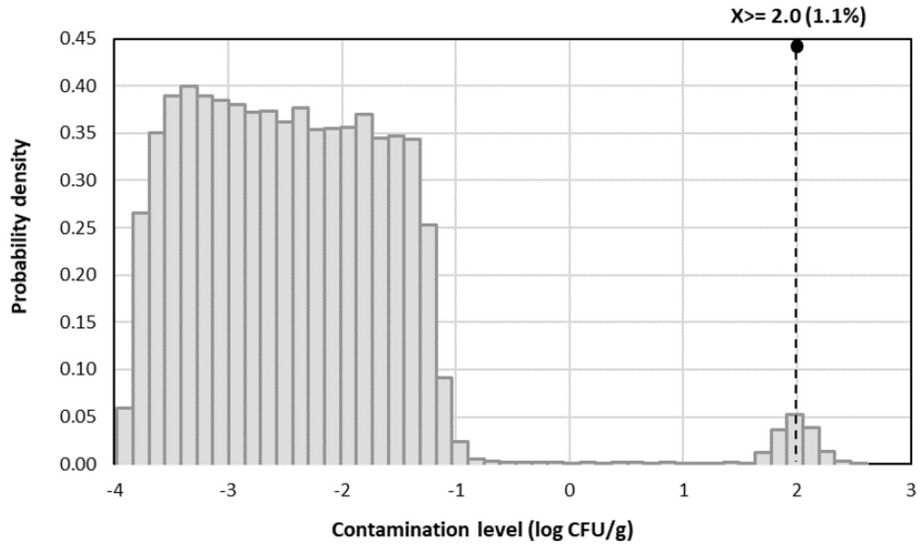

Fig. 4. Predicted distributions of levels of *L. monocytogenes* (log CFU/g) on Coppa di Testa at the point of industry; From the plot the proportion of samples containing >100 CFU/g is estimated to be ~1.1 %.

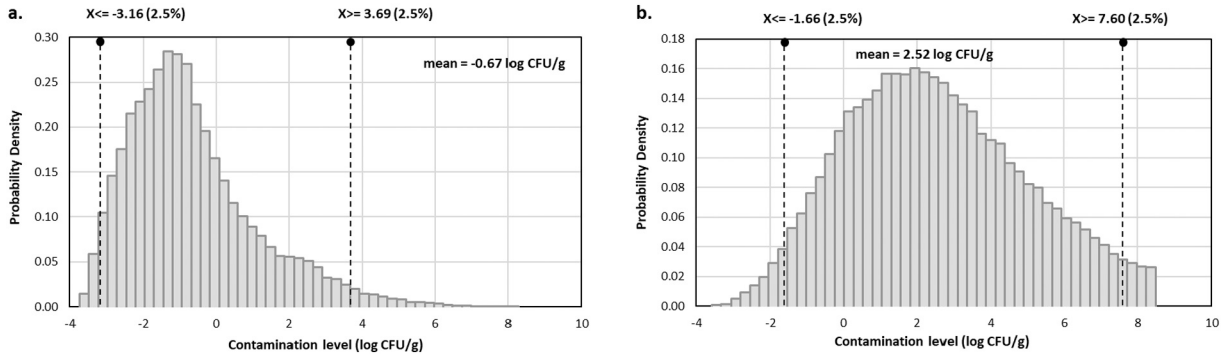

Fig. 5. Predicted distributions of levels of *L. monocytogenes* (log CFU/g) on Coppa di Testa at the point of consumption, purchased as either (a) open/sliced or (b) vacuum-packed. The proportion of samples containing >100 CFU/g is estimated to be 9.3 % for (a) and 55 % for (b), respectively.

Table 7
Output of QMRA model regarding the baseline scenario.[a]

	Product purchased vacuum-packed		Product purchased open/sliced	
	General population	High-risk population	General population	High-risk population
Risk of listeriosis per serving				
Mean	2.44×10^{-6}	1.09×10^{-4}	1.35×10^{-8}	6.18×10^{-7}
SD	1.80×10^{-5}	8.01×10^{-4}	6.49×10^{-7}	2.90×10^{-5}
Minimum	0.00	0.00	0.00	0.00
5th percentile	2.3×10^{-14}	1.06×10^{-12}	0.00	0.00
Median	9.66×10^{-11}	4.32×10^{-9}	4.74×10^{-14}	2.12×10^{-12}
95th percentile	3.95×10^{-6}	1.76×10^{-4}	3.69×10^{-10}	1.65×10^{-8}
Maximum	5.01×10^{-4}	2.22×10^{-2}	7.63×10^{-5}	3.40×10^{-3}
Number of listeriosis cases in a population of 100,000				
Mean	0.24	10.90	0.001	0.06
SD	1.87	80.39	0.083	2.92
Minimum	0	0	0	0
5th percentile	0	0	0	0
Median	0	0	0	0
95th percentile	0	18	0	0
Maximum	49	2179	9	341

[a] Baseline scenario considers consumption of any serving regardless of initial contamination and product shelf life up to 180 days at the retail. Based on 100,000 iterations using Monte Carlo simulation.

contamination levels at consumption (Fig. 5), thus further increasing the resulting maxima in the output of the model, provided the assumptions described above. Therefore, a possible intervention measure would be to shorten the shelf-life for vacuum-packed products to enable the producer to achieve the criterion of 100 CFU/g in the absence of preservatives. The effect of storage time on the risk of listeriosis and number of cases is further investigated in the alternative scenarios below.

3.2.2. Evaluation of alternative scenarios

Two scenarios for assessing potential interventions to reduce the relative risk of listeriosis due to consumption of Coppa di Testa were investigated: i) setting a shorter shelf-life for the product and ii) improving the temperature of domestic storage. Considering each scenario separately, the corresponding input variables were modified and the model was re-run.

Currently, the use-by-date for vacuum-packed Coppa di Testa products varies broadly from 30 to 180 days. Furthermore, a use-by-date for products opened and sliced at the retail or opened at home is not applied. Based on our consumer survey, after opening, vacuum-packed products are consumed within 1.7–3.4 days, however Ross et al., reported that opened products may occasionally be held for 10 or 15 days before use, despite evident deterioration (Ross et al., 2009). Therefore, in the first scenario, a maximum shelf-life (use-by-date) of 30 days was set, since Coppa di Testa is very often sold within one month of production despite the application of longer use-by-dates (expert advice) and closer to industry reports concerning other RTE meat products (Mataragas et al., 2010). This scenario reduced the mean listeriosis cases for both the general and high-risk populations (Tables 8 & 9). The relative risks decreased to ~33 % and ~85 % of the baseline scenario for the vacuum-packed and open/sliced products, respectively.

In the second scenario, the temperature of domestic refrigerators was improved by modifying the normal distribution variables N(8.81;2.91) °C obtained from experimental results to N(5.0; 1.5) °C, thus reducing the mean temperature. This intervention scenario reduced the risks of listeriosis for the longer-stored vacuum-packed product by ~1 log or ~80 % compared to the baseline scenario. The reduction in mean cases in the population is displayed in Tables 8 & 9. Lowering the domestic refrigerator temperature reduced the mean *L. monocytogenes*

Table 8
Predicted listeriosis cases[a] in the general population related with the consumption of Coppa di Testa in Italy and the impact of potential interventions.

	Baseline scenario (no intervention)		Use by date maximum 30 days (scenario 1)		Improving domestic refrigeration (scenario 2)	
	VP	O/S	VP	O/S	VP	O/S
Mean	0.24	0.001	0.16	0.0001	0.052	0.005
SD	1.87	0.083	1.47	0.016	0.83	0.150
Median	0	0	0	0	0	0
95th percentile	0	0	0	0	0	0

[a] Predicted for 100,000; VP: vacuum-packed; O/S: open/sliced.

Table 9
Predicted listeriosis cases[a] in the high-risk population related with the consumption of Coppa di Testa in Italy and the impact of potential interventions.

	Baseline scenario (no intervention)		Use by date maximum 30 days (scenario 1)		Improving domestic refrigeration (scenario 2)	
	VP	O/S	VP	O/S	VP	O/S
Mean	10.9	0.06	7.28	0.01	2.33	0.21
SD	80.39	2.92	63.74	0.54	37.06	5.35
Median	0	0	0	0	0	0
95th percentile	18	0	7	0	0	0

[a] Predicted for 100,000; VP: vacuum-packed; O/S: open/sliced.

concentration in the vacuum-packed product from 2.5 log CFU/g in the baseline scenario to 0.5 log CFU/g. The population of the pathogen in the open/sliced product was not affected by this intervention scenario and neither were the mean estimated cases (Tables 8 & 9). Therefore, we concluded that lowering the domestic storage temperature could contribute to a reduction of listeriosis cases only caused by vacuum-packed products, which reportedly are stored longer in domestic refrigerators than open/sliced products. Sensitivity analysis of the QMRA reveals the most significant parameters influencing the final risk estimation in either vacuum-packed and open/sliced products. From Fig. 6b, the "temperature at domestic storage" emerges as a crucial parameter influencing the risk of listeriosis in the vacuum-packed product and as a less important parameter in the open/sliced product. The "storage time at retail" parameter, closely related to the use-by-date of the product, affects both types of packaged head cheese and its reduction, decreases the risk of listeriosis as reflected in the predicted cases. Similar findings on the effect of reducing storage time and temperature on listeriosis cases were previously reported for various RTE meat products (EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2018; Tsaloumi et al., 2021). From the results of this study, it can be concluded that consumer awareness and implementation of good practices in the domestic storage of RTE head cheese, can significantly reduce the risk of listeriosis. Even though both intervention scenarios provide effective ways to reduce risk, efforts should be made to minimize the microbial load at industry level - a major risk coefficient - by preventing product contamination due to human handling and contact with manufacturing equipment.

4. Conclusions

The present quantitative risk assessment is the first attempt to estimate the listeriosis risk related to the consumption of Coppa di Testa, a traditional RTE head cheese produced in Italy. The probabilistic model developed in @Risk software predicts the risk along two different paths: a product purchased as vacuum-packed and a product purchased as sliced/opened at the retail. Given the assumptions and data inputs in the model, the QMRA predicted the listeriosis risk to range from 10^{-4} to 10^{-8} (mean), depending on product packaging and population of concern (general or high-risk). The results support the idea that vacuum-packed products present higher risk of listeriosis compared to open/

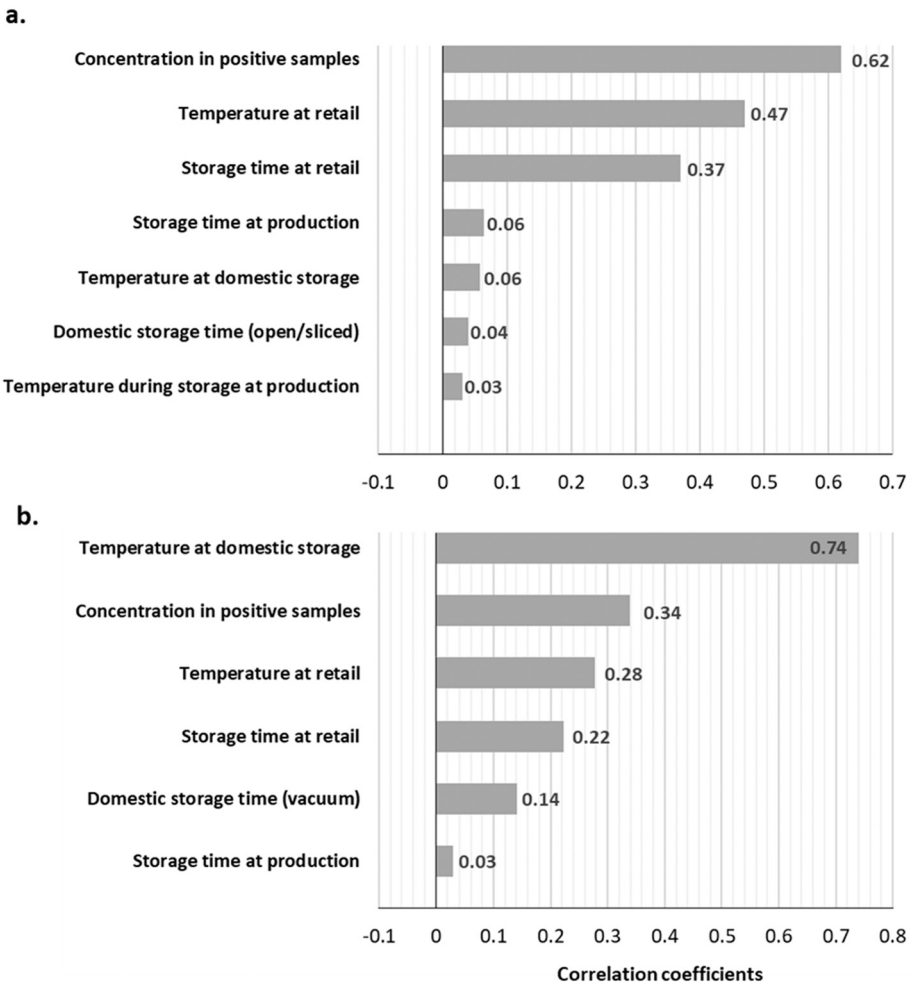

Fig. 6. Sensitivity analysis showing the correlation coefficients of the most important factors contributing to the model outputs for (a) open/sliced or (b) vacuum-packed product.

sliced products due to their longer storage and this risk is higher for vulnerable populations (mean risk at the range of 10^{-4}). Efforts to reduce storage time or better control temperature at domestic storage will reduce the risk. The latter was concluded after identifying important factors contributing to listeriosis cases and the application of two "what-if" scenarios, which evaluated the effect of potential mitigation strategies at both the retail and consumer levels. The risk associated with consumption of vacuum-packed product decreased by either scenario – lowering the domestic temperature and limiting the use-by-date to 30 days – by 80 % and 33 %, respectively. This reduction in risk was observed to affect both consumer populations. Listeriosis risk from the sliced/opened product was only reduced by limiting the shelf-life. As the latter parameter is crucial for ensuring the safety of both vacuum-packed and sliced/opened products, raising consumer awareness – especially among the vulnerable – is suggested as a mitigation measure. The model could be further explored to assess additional scenarios such as lowering the contamination at industry level. Due to absence of data, the model is limited by some assumptions, including the absence of cross-contamination at the retail, the lack of antimicrobial factors and other factors affecting the growth of the pathogen (i.e. lactic acid bacteria) in the product. Nevertheless, it constitutes a first baseline approach for better risk management of the Italian head cheese.

Funding

This paper was financially supported by a European Food Safety Authority EU-FORA action grant [GP/EFSA/AFSCO/2017/08-GA16]; title: Risk Assessment for *Listeria monocytogenes* in Ready to Eat Food.

Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this article.

References

Anonymous, 2005. Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Off. J. Eur. Union 1–26.
Anonymous, 2007. Commission Regulation (EC) No. 1441/2007 of 5 December 2007 an amendment of the Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs. Off. J. Eur. Union 12–29.
Bardasi, L., Bonilauri, P., Rugna, G., Galletti, G., Fedrizzi, G., Santandrea, G., Gandolfi, P., Vecchi, G., Merialdi, G., 2010. Growth of naturally occuring listeria innocua in coppa di testa. Ital. J. Food Saf. 1, 35–39.
Bassett, J., Nauta, M., Lindqvist, R., Zwietering, M., 2012. Tools for microbiological risk assessment. In: ILSI Europe Report Series.
Buchanan, R.L., Damert, W.G., Whiting, R.C., van Schothorst, M., 1997. Use of epidemiologic and food survey data to estimate a purposefully conservative dose-response relationship for listeria monocytogenes levels and incidence of listeriosis (†). J. Food Prot. 60, 918–922.
Buchanan, R.L., Gorris, L.G.M., Hayman, M.M., Jackson, T.C., Whiting, R.C., 2017. A review of listeria monocytogenes : an update on outbreaks, virulence, dose-response, ecology, and risk assessments. Food Control 75, 1–13.
Campagnollo, F.B., Gonzales-Barron, U., Pilão Cadavez, V.A., Sant'Ana, A.S., Schaffner, D.W., 2018. Quantitative risk assessment of Listeria monocytogenes in traditional Minas cheeses: the cases of artisanal semi-hard and fresh soft cheeses. Food Control 92, 370–379.
Cenci-Goga, B.T., Selvaggini, R., Corry, J.E.L., 2002. Time/Temperature Recording With Data-loggers and Development of Specific Software for Cumulative Abuse Measurement 48° ICoMST, Rome, Italy.
Centers for Disease Control and Prevention (CDC), 2011. Outbreak of invasive listeriosis associated with the consumption of hog head cheese–Louisiana, 2010. MMWR 60, 401–405.
Condoleo, R., Mezher, Z., Marozzi, S., Guzzon, A., Fischetti, R., Senese, M., Sette, S., Bucchini, L., 2017. Risk assessment of human listeriosis from semisoft cheeses made from raw sheep's milk in Lazio and Tuscany (Italy). Risk Anal. 37, 661–676.
Duranti, A., Sabbatucci, M., Blasi, G., Acciari, V.A., Ancora, M., Bella, A., Busani, L., Centorame, P., Camma, C., Conti, F., De Medici, D., Di Domenico, M., Di Marzio, V., Filippini, G., Fiore, A., Fisichella, S., Gattuso, A., Gianfranceschi, M., Graziani, C., Guidi, F., Marcacci, M., Marfoglia, C., Neri, D., Orsini, M., Ottaviani, D., Petruzzelli, A., Pezzotti, P., Rizzo, C., Ruolo, A., Scavia, G., Scuota, S., Tagliavento, G., Tibaldi, A., Tonucci, F., Torresi, M., Migliorati, G., Pomilio, F., 2018. A severe outbreak of listeriosis in Central Italy with a rare pulsotype associated with processed pork products. J. Med. Microbiol. 67, 1351–1360.
EFSA (European Food Safety Authority), 2018. Technical Report of the Outcome of the Public Consultation on the Draft Scientific Opinion on Listeria Monocytogenes Contamination of Ready-to-eat Foods and the Risk for Human Health in the EU. EFSA Supporting Publication 15, EN-1352, 1367pp.
EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2018. Scientific opinion on the Listeria monocytogenes contamination of ready-to-eat foods and the risk for human health in the EU. EFSA J. 16 (5134), 5173 pp.
European Centre for Disease Prevention and Control (ECDC), 2018. Listeria. In: ECDC. Annual Epidemiological Report for 2016. ECDC, Stockholm.
European Centre for Disease Prevention and Control (ECDC), 2020. Listeriosis. In: ECDC. Annual Epidemiological Report for 2017. ECDC, Stockholm.
Food and Agriculture Organization of the United Nations, World Health Organization (FAO/WHO), 2004. Risk assessment of Listeria monocytogenes in ready-to-eat foods. Microbiological risk assessment series 5. Available at: http://www.fao.org/3/y5394e/y5394e00.htm.
Food and Drug Administration (FDA)/Health Canada—Santé Canada, 2015. Quantitative assessment of the risk of listeriosis from soft-ripened cheese consumption in the United States and Canada: interpretative summary. Available at: https://www.fda.gov/food/cfsan-risk-safety-assessments/joint-fda-health-canada-quantitative-assessment-risk-listeriosis-soft-ripened-cheese-consumption.
Giovannini, A., Migliorati, G., Prencipe, V., Calderone, D., Zuccolo, C., Cozzolino, P., 2007. Risk assessment for listeriosis in consumers of Parma and san daniele hams. Food Control 18, 789–799.
Gogou, E., Katsaros, G., Derens, E., Alvarez, G., Taoukis, P.S., 2015. Cold chain database development and application as a tool for the cold chain management and food quality evaluation. Int. J. Refrig. 52, 109–121.
Gray, J.A, Chandry, P.S., Kaur, M., Kocharunchitt, C., Bowman, J.P., Fox, E.M., 2018. Novel biocontrol methods for *Listeria monocytogenes* biofilms in food production facilities. Front Microbiol. https://doi.org/10.3389/fmicb.2018.00605.
Hadjicharalambous, C., Grispoldi, L., Goga, B.C., 2019. Quantitative risk assessment of Listeria monocytogenes in a traditional RTE product. EFSA J. 17 (S2), e170906, 9 pp.
Jofré, A., Latorre-Moratalla, M.L., Garriga, M., Bover-Cid, S., 2019. Domestic refrigerator temperatures in Spain: assessment of its impact on the safety and shelf-life of cooked meat products. Food Res. Int. 126, 108578.
Koutsoumanis, K., Pavlis, A., Nychas, G.J., Xanthiakos, K., 2010. Probabilistic model for listeria monocytogenes growth during distribution, retail storage, and domestic storage of pasteurized milk. Appl. Environ. Microbiol. 76, 2181–2191.
Koutsoumanis, K.P., Aspridou, Z., 2016. Moving towards a risk-based food safety management. Curr. Opin. Food Sci. 12, 36–41.
Kurpas, M., Wieczorek, K., Osek, J., 2018. Ready-to-eat meat products as a source of listeria monocytogenes. J. Vet. Res. 62, 49–55.
Lammerding, A.M., Fazil, A., 2000. Hazard identification and exposure assessment for microbial food safety risk assessment. Int. J. Food Microbiol. 58, 147–157.
Mataragas, M., Drosinos, E.H., Siana, P., Skandamis, P., Metaxopoulos, I., 2006. Determination of the growth limits and kinetic behavior of listeria monocytogenes in a sliced cooked cured meat product: validation of the predictive growth model under constant and dynamic temperature storage conditions. J. Food Prot. 69, 1312–1321.
Mataragas, M., Zwietering, M.H., Skandamis, P.N., Drosinos, E.H., 2010. Quantitative microbiological risk assessment as a tool to obtain useful information for risk managers–specific application to listeria monocytogenes and ready-to-eat meat products. Int. J. Food Microbiol. 141 (Suppl. 1), S170–S179.
Matle, I., Mbatha, K.R., Madoroba, E., 2020. A review of listeria monocytogenes from meat and meat products: epidemiology, virulence factors, antimicrobial resistance and diagnosis. Onderstepoort J. Vet. Res. 87 e1-e20.
McLauchlin, J., Mitchell, R.T., Smerdon, W.J., Jewell, K., 2004. Listeria monocytogenes and listeriosis: a review of hazard characterisation for use in microbiological risk assessment of foods. Int. J. Food Microbiol. 92, 15–33.
Mejlholm, O., Dalgaard, P., 2015. Modelling and predicting the simultaneous growth of listeria monocytogenes and psychrotolerant lactic acid bacteria in processed seafood and mayonnaise-based seafood salads. Food Microbiol. 46, 1–14.
Mercier, S., Villeneuve, S., Mondor, M., Uysal, I., 2017. Time-temperature management along the food cold chain: a review of recent developments. Compr. Rev. Food Sci. Food Saf. 16, 647–667.
Orsi, R.H., den Bakker, H.C., Wiedmann, M., 2011. *Listeria monocytogenes lineages*: genomics, evolution, ecology, and phenotypic characteristics. Int. J. Med. Microbiol. 301, 79–96.
Orsi, R.H., Wiedmann, M., 2016. Characteristics and distribution of listeria spp., including listeria species newly described since 2009. Appl. Microbiol. Biotechnol. 100, 5273–5287.
Ratkowsky, D.A., Olley, J., McMeekin, T.A., Ball, A., 1982. Relationship between temperature and growth rate of bacterial cultures. J. Bacteriol. 149, 1–5.
Roccato, A., Uyttendaele, M., Membré, J.M., 2017. Analysis of domestic refrigerator temperatures and home storage time distributions for shelf-life studies and food safety risk assessment. Food Res. Int. 96, 171–181.
Ross, T., Rasmussen, S., Fazil, A., Paoli, G., Sumner, J., 2009. Quantitative risk assessment of listeria monocytogenes in ready-to-eat meats in Australia. Int. J. Food Microbiol. 131, 128–137.
Szczawiński, J., Ewa Szczawińska, M., Łobacz, A., Tracz, M., Jackowska-Tracz, A., 2017. Modelling the growth rate of listeria monocytogenes in cooked ham stored at different temperatures. J. Vet. Res. 61, 45–51.
Tsaloumi, S., Aspridou, Z., Tsigarida, E., Gaitis, F., Garofalakis, G., Barberis, K., Tzoumanika, F., Dandoulaki, M., Skiadas, R., Koutsoumanis, K., 2021. Quantitative risk assessment of listeria monocytogenes in ready-to-eat (RTE) cooked meat products sliced at retail stores in Greece. Food Microbiol. 99, 103800.
U.S. Food and Drug Administrator (FDA), U.S. Department of Agriculture (USDA), 2003. Quantitative assessment of the relative risk to public health from foodborne Listeria

monocytogenes among selected categories of ready-to-eat foods. https://www.fda.gov/food/foodscienceresearch/risksafetyassessment/ucm183966.htm.