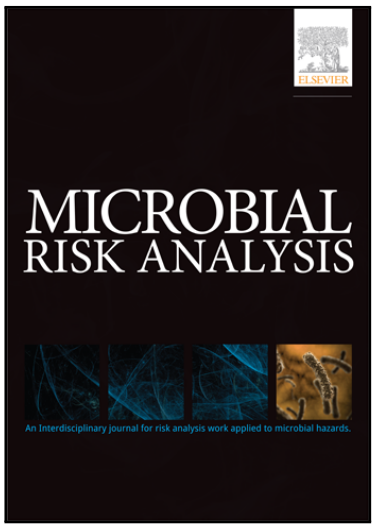

Microbial risk and health burden associated with the domestic preparation of lentils in France and Hungary

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Highlights:

- Assessment of risk with home-cooked lentil consumption in France and Hungary
- *B. cereus* and *L. monocytogenes* as microbial hazards in home-prepared lentils
- A probabilistic model with uncertainty and variability was developed using 2D Monte Carlo methods
- Low risk from proper lentil consumption practices, but may increase with new consumption patterns
- Need for QMRA before pushing new diets, ingredients or plant-based alternatives

Microbial risk and health burden associated with the domestic preparation of lentils in France and Hungary

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Abstract:

Lentils are promoted as an alternative protein source due to their agricultural and nutritional benefits. However, information on the microbial risks associated with lentil consumption in domestic settings is limited. The countries of France and Hungary were selected to represent the lentil consumption in two different supply chains. *Bacillus cereus* was identified as a pathogen of concern in both hot and cold dishes, whereas *Listeria monocytogenes* was only identified in cold dishes. A probabilistic model accounting for uncertainty and variability was constructed, estimating the microbial concentration at subsequent stages of domestic handling: cooking, cooling, and 24–96 h of chilled storage. The number of foodborne illness cases and daily adjusted life years (DALY) were estimated at the point of consumption.

The results were analysed at each stage; for example, at 96 h, *B. cereus* showed mean values of 2.52 [1.75; 4.12] log CFU/g in France and 2.03 [1.24; 3.56] log CFU/g in Hungary. For *Listeria monocytogenes*, the mean estimates were lower at 0.48 [−0.20; 1.16] log CFU/g in France and 0.69 [0.07; 1.36] log CFU/g in Hungary. The overall number of foodborne illness cases from both pathogens was computed based on the consumption frequency. They were estimated to be 0 [0–44] cases per 100,000 in France and 0 [0–5.8] in Hungary.

The uncertainty intervals are relatively large, reflecting uncertainty in the estimates, meaning that the risk is not an absolute zero. Moreover, it is likely that dietary shifts towards less meat consumption, as promoted by various institutions, will occur. Extended batch cooking practices can pose an additional risk of foodborne illnesses in the future.

Keywords: *Bacillus cereus*, *Listeria monocytogenes*, DALY, leguminous, domestic practices, food safety, Monte Carlo

1. Introduction

Lentils are seen as a key crop of the future due to their agricultural and nutritional benefits. In terms of agriculture, they are known for their nitrogen fixation activity and ability to tolerate dry conditions. Their nutritional role has been reported to contribute to consumers' protein, vitamins, and mineral requirements (Conti et al., 2021; Iqbal et al., 2006; Romano et al., 2021). Therefore, lentils are positioned as a sustainable protein alternative in several proposed agroecological models (e.g., Afterres2050, Transition 2050, and TYFA 2050). These models are characterised by an increased consumption of non-animal protein (i.e., legumes) and a reduction in red meat (i.e., bovine) and, to a lesser extent, monogastric (i.e., chicken and pork) livestock (Billen et al., 2021; Duru and Therond, 2023). This shift from animal production will enable a focus on farming crops that have lower greenhouse gas emissions and preserve wildlife biodiversity. As part of regional policies, several European projects (e.g., Pulse Increase, legume generation, and smart protein project) and campaigns have been launched to encourage farmers to adopt this crop and people to increase lentil consumption in their diet. These trends have been observed in the European region, including in France and Hungary (e.g., European Union (EU) green deal and Cap proteins) (European Commission, 2021, 2020). However, this wide-range push towards dietary shifts is not without risks. Several studies have called for an analysis of the associated chemical, nutritional, and biological risks with newer trends and dietary patterns (Eygue et al., 2020; Guillier et al., 2016; Poissant et al., 2023).

Lentils are a major crop in Canada, India, and Australia, which are the top producers. In the EU, they are imported from Canada, Turkey, and the USA, while local production is led by Italy and France. The consumption pattern of lentils varies across the world, with the highest in Asian countries (e.g., India), where it is the key protein source. However, in the EU, lentil consumption remains low with an increasing trend, particularly in France and Hungary (Mombert et al., 2024; Nagy et al., 2021). Lentil production increased from 75,0000 (2017) tonnes to 116,000 (2021), with France and Spain as the leading producers. The import volume of lentils ranged from 246,000 to 230,000 tonnes between 2017 and 2021 (Centre for the Promotion of Imports, 2023). This can also be seen with lentil-dedicated farmlands in Europe, which peaked around 2018 but declined after 2020 (Terres Univia, 2022).

In these two countries, lentils are mostly consumed in domestic settings and prepared from dried lentils, followed by canned ones, making these their top market forms. The domestic preparation of dried lentils is done by batch cooking, and the leftovers are stored in the refrigerator until finished. In France, lentils are mostly prepared as a hot dish or cold salad dish, which is then mixed with other types of food. In Hungary, they are mostly prepared as hot soup or cold sandwich spreads (Nagy et al., 2021; Yabré and Membré, 2022).

Dietary shifts can provide benefits for meeting nutritional requirements but can also cause exposure to food safety hazards, which can have implications on the health burden of consumers (Pires et al., 2020). An example is plant-based milk alternatives, which can meet protein and energy requirements (Craig et al., 2023) but can increase exposure to heavy metals and mycotoxins (Santillana Farakos et al., 2021). Dietary studies exploring current and alternative legume-based diets paint the same picture (Ferreira et al., 2023; Mihalache et al., 2024). In a nutrition intervention study, Ferreira et al. (2023) demonstrated that legumes did not aggravate nutritional deficiency, and there was a slight decline in vitamin B12. However, Mihalache et al. (2024) found that a legume-based diet reduced the negative impacts of red and processed meats but also posed challenges in meeting micronutrient (e.g., Fe and Se) needs and increased exposure to heavy metals (e.g., arsenic, lead, and cadmium) and mycotoxins (AFB1). Therefore, several studies have called for a more systematic assessment of novel dietary patterns (Eygue et al., 2020; Santillana Farakos et al., 2021).

Assessing the impacts of food systems is a crucial prerequisite for promoting food consumption patterns and diets. To add complexity is the recognition that other impacts of food systems extend beyond consumer health. Therefore, there is a potential for contradictions between different food policies that shift diets that must be reconciled (e.g., sustainability and food safety) (Guillier et al., 2016). This can occur in the context of climate mitigation, where food safety measures can be added to food production activity. However, this may contribute to environmental impact via greenhouse gas emissions. As such, win–win solutions are can be achieved through assessments (i.e., lifecycle assessments and quantitative microbial risk assessments) (Feliciano et al., 2022). This indicates the need for different analytical tools that allow compromise without sacrificing human health (Guillier et al., 2016). Therefore, a holistic risk assessment framework has been proposed to incorporate tools that consider several dimensions when assessing food systems (e.g., economic, environmental, health, and nutrition) (Országh et al., 2024).

Food handling and cooking practices can be vulnerable to microbial contamination (Byrd-Bredbenner et al., 2013; Fischer et al., 2007). Pathogens in raw meat (e.g., *Escherichia coli*, *Campylobacter jejuni*) can be cross-contaminated in the kitchen area through contaminated hands, chopping boards, and knives during food preparation (Kennedy et al., 2011). Several foodborne outbreaks have been linked to homes in different countries due to these contamination events (Redmond and Griffith, 2003). This highlights the role of home and existing practices, cooking conditions, and refrigeration in food safety. Therefore, modelling and capturing the conditions at home must be included in the risk assessment of the consumption. This can aid in understanding microbial risk and the possible control points.

Microbial risk assessment is a tool that can be used for risk management by identifying the vulnerable segments of the supply chain (FAO and WHO, 2021). This can be performed by determining which practices or segments enable pathogen growth. Ultimately, food safety managers can aid in the design of products to limit microbial growth, and regulators or policymakers can determine whether dietary trends or shifts to new food products can pose foodborne microbiological risks. Given the increasing trend of lentil consumption and existing domestic practices, an assessment of risk and its possible burden on the population is still missing. Therefore, the current study aimed to estimate the health burden associated with the consumption and domestic preparation of lentils in France and Hungary. These countries were chosen to study lentil consumption due to their similar cooking practices (hot and cold dishes) and representation of two different supply chains. France reflects a supply chain that both produces and imports, whereas Hungary is a mostly importing country. This study applied a probabilistic modelling approach that incorporated consumer practices and data available in the literature. In addition, the results were expressed in terms of the total burden of diseases from the projected annual cases. This research aims to demonstrate the added value of risk assessments in informing the associated risks that emerge from increased lentil consumption.

2. Materials and Methods:

2.1. Identification of microbial hazards in lentils and domestic preparation

The current study assessed the microbial risks associated with home-cooked lentil dishes in France and Hungary. In these countries, lentils are prepared in domestic settings from dried and canned lentils, making these the top market forms (Terres Univia, 2024). Lentils are prepared differently between the countries, as lentil salad in France and soup in Hungary, with differences in cooking duration. Nevertheless, consumers follow a similar batch-cooking approach, and leftovers are stored for consumption. Leftovers are usually reheated and eaten hot, but some are eaten cold, as in salads.

Hazard identification was performed following the methodology described in the literature (Codex Alimentarius Commission, 1999). It was assumed that food safety management systems and prerequisite programs were implemented during raw lentil production. The list of possible microorganisms of concern was made by drawing on those associated with lentil products as reported by food rapid alert systems (e.g., EURASSFF, USFDA, Rappelconso.fr, and Nebih) and food microbiology literature (Anses, 2022; ICMSF, 2005). These microorganisms were then contextualised based on their possible entry points along the lentil supply chain (e.g., *Listeria monocytogenes* recontamination during cold storage) (**Supplementary Table 1**). Subsequently, a series of filtering steps was performed by selecting which microorganisms can survive at the point of consumption. First, most of the vegetative pathogens identified at the initial part of the supply chain were eliminated during the canning of lentils or assumed to be reduced significantly during batch-cooking at home and, thus, do not pose microbiological risks. Similarly, foodborne viruses that cannot propagate along the supply chain were not considered in this study. In contrast, at this stage, the spores of *Clostridium botulinum*, *C*. *perfringens*, and *Bacillus cereus* were retained. These spore formers may survive the conditions experienced by dried lentils and during cooking and may germinate during the storage of cooked lentils. However, *C*. *botulinum and C*. *perfringens* were removed, given that they are obligate anaerobes and are not relevant at domestic stages.

Consumers were generally considered to respect good hygiene practices, including cooling for a short period at ambient temperature. However, recontamination events can introduce *L*. *monocytogenes* and *Staphylococcus aureus* after batch cooking, cooling, and subsequent storage. *Staphylococcus aureus* requires a prolonged storage period at ambient temperatures to enable population growth and toxin production. In a previous survey and as reflected in this study, lentils are stored at room temperature after cooling for a limited period. The leftovers are kept in the refrigerator and reheated or consumed cold until the entire batch is finished. *Listeria monocytogenes* was selected over other pathogens, as it grows well at refrigeration temperatures. However, recontamination events after batch cooking, cooling, and

subsequent storage may enable *L*. *monocytogenes* to pose a risk if the lentil dish is eaten cold, as in a salad or spread. This pathogen poses a threat by being able to propagate during cold storage.

Therefore, two pathogenic microorganisms were retained for microbial risk assessment, namely *B*. *cereus* and *L*. *monocytogenes*. These two are considered to pose microbiological risks given the domestic practices before consumption. Furthermore, *Bacillus cereus* was further specified in this study as a microbial hazard by selecting two (groups III and IV) of the seven groups (Guinebretière et al., 2010). This is due to their ability to grow at room temperature, pathogenicity, and prevalence in being implicated in foodborne disease outbreaks (Anses, 2021; Glasset et al., 2016).

2.2. Lentil consumption data and domestic practices of the French and Hungary populations

French consumption data were collected from the third French Individual and National Food Consumption Survey (INCA3) conducted by the French Food Safety Agency (Dubuisson et al., 2019). This survey was carried out in February 2014 and September 2015 with 5855 individual participants. Among these were 284 individuals who declared that they consumed canned and dried lentils (150 young and 134 adults). The Hungarian consumption data were collected from the Hungarian national food consumption survey of the EUMENU project (National Food Chain Safety Office Hungary et al., 2020). This survey was conducted from 2018 to 2020 with 1585 participants, of whom 87 consumed lentils.

These consumption data were used to estimate portion sizes. A non-parametric bootstrap procedure was carried out to capture the uncertainty. Their variability was described through gamma and Weibull distributions in France and Hungary, respectively. The domestic practices were determined from an online survey conducted among 556 lentil consumers in France (Yabré and Membre, 2022). This survey contained information on the frequency of lentil consumption (1–5 times per week) and consumption practices (e.g., leftover frequency, cooling, and storage duration). The leftover frequency and storage duration in Hungary were estimated from Koppel et al. (2016). The domestic practices and lentil consumption data used in the current study are summarised in **Table 1**.

2.3. Exposure model frameworks for *Listeria monocytogenes* and *Bacillus cereus*

2.3.1. Model framework overview

The microbial risk assessment models were constructed for *L*. *monocytogenes* and *B*. *cereus* for both hot and cold lentil dishes in France and Hungary. These models followed a modular modelling approach (Nauta, 2001). The modules included initial contamination of raw dry lentils, bacterial destruction during cooking, growth during cooling or refrigeration, consumption, and risk estimation modules. The main difference between the models is the contamination pathway. *Bacillus cereus* contamination was assumed to start from spores in raw lentils that resist inactivation during cooking and grow during storage. Through the toxin produced during storage, *B*. *cereus* poses a risk to both heated and cold-served lentil leftovers. However, *L*. *monocytogenes* occurs through recontamination during the cooling and storage phase, posing a risk to cold leftovers. It was not considered for hot-served dishes due to inactivation during the reheating step. The inputs used in the model and specific conditions applied between France and Hungary are listed in **Table 1**.

2.3.2. Bacillus cereus exposure assessment model

2.3.2.1. Module 1: Initial contamination and prevalence of B. cereus in lentils

The initial counts of *B*. *cereus* were taken from the reported spore concentration levels and prevalence among legume samples (i.e., red lentils and yellow split beans) (Blakey and Priest, 1980). In their study, the detection threshold was set to 2 log CFU/g, which explains why this value appears as the threshold in our study, modelled as a Bernoulli. The maximum level of *B*. *cereus* reported in their paper was 45,000 CFU/g, which we used as our upper bound. This information was used to construct the concentration levels as greater than 2 $\log_{10}$ ($N0X^{Bc>2}$) or less than 2 $\log_{10}$ CFU($N0X^{Bc<2}$). The uncertainty and variability associated with these concentration levels were captured using a Pert distribution. These values were expressed per portion size (**Eq. 1a**) and as the number of bacterial cells (**Eq. 1b**).

$$log_{10}\, N^{Bc<2} = Pert\ (Min; Most\ likely; Max) \begin{Bmatrix} min: 0.3 + \log_{10}\text{portion size} \\ mode: 1 + \log_{10}\text{portion size} \\ max: 1.95 + \log_{10}\text{portion size} \end{Bmatrix}$$

$$log_{10}\, N^{Bc>2} = Pert\ (Min; Most\ likely; Max) \begin{Bmatrix} min: 2 + \log_{10}\text{portion size} \\ mode: 2.3 + \log_{10}\text{portion size} \\ max: 3 + \log_{10}\text{portion size} \end{Bmatrix}$$

(1a)

$$N^{Bc<2} = 10^{log_{10} N0 X^{Bc<2}} \quad \text{and } N^{Bc>2} = 10^{log_{10} N0 X^{Bc>2}}$$

(1b)

The initial contamination and prevalence of contamination were translated into different packs used for cooking, representing different homes. The modelling strategy was initiated by assigning the log contamination levels in the total lentil packs per year and then by representing the probability of one pack being contaminated.

The total prevalence of *B. cereus* was captured by reflecting the uncertainties surrounding the contamination levels using a beta distribution (**Eq. 2a**) with the number of samples containing greater than 2 $\log_{10}$ CFU ($N^{Bc>2}$) and less than 2 $\log_{10}$ CFU ($N^{Bc<2}$) (s) and the total number of samples (n) tested (Blakey and Priest, 1980). The variability of contamination levels was computed using the Bernoulli distribution (**Eq. 2b**).

$$P_{t0}^{Bc} = Beta\ (s+1, n-s+1)$$

(2a)

$$P_{Bc\ contam} = Bernouilli\ (P_{t0}^{Bc})$$

(2b)

The percentage of contaminated packs with less than 2 $\log_{10}$ CFU was described using a beta distribution (**Eq. 3a**) with the number of samples containing less than 2 $\log_{10}$ CFU ($N^{Bc<2}$) (s) and number of samples containing greater than 2 $\log_{10}$ CFU ($N^{Bc>2}$) (n) described (Blakey and Priest, 1980). The variability of *B. cereus* contamination in the lentil packs was captured using the Bernoulli distribution (**Eq. 3b**).

$$\%\ contam\ packs < 2 = Beta\ (s+1, n-s+1)$$

(3a)

$$P_{contam_{pack}<2} = Bernouilli\ (\%\ contam\ packs < 2)$$

(3b)

The total prevalence of *B. cereus* in lentil packs and its associated levels were computed (**Eq. 4**) and expressed as the quantity of *B. cereus* (**Eq. 5**).

$$N0_{total_{Bc}} = N^{Bc<2} x P_{contam_{pack}<2} + \left(1 - P_{contam_{pack}<2}\right) x\ N^{Bc>2}$$

(4)

$$Q_{t0}^{Bc} = N0_{total_{Bc}} x P_{Bc\ contam}$$

(5)

Bacillus cereus was estimated per strain, namely groups III and IV. This study focused on groups III and IV, as they are known to be the main toxin producers in the *B. cereus* group (Guinebretière et al., 2008). Due to the lack of data on their proportions in lentil matrices, we assumed a variable distribution ranging from 20 to 80% between these groups (**Eq. 6**). The estimations were reflected in a uniform distribution given an absence of literature data on the exact strain prevalence in lentils (**Eq. 7**).

$$P_{GIII}^{Bc} = Uniform\ (0.20, 0.80) \text{ and } P_{GIV}^{Bc} = (1 - P_{GIII}^{Bc})$$

(6)

$$N_0^{Bc\ III\ or\ IV} = Q_{t0}^{Bc} x\ P_{G\ III\ or\ IV}^{Bc}$$

(7)

2.3.2.2. Module 2: Microbial inactivation during batch cooking of lentils

The current module was built by modelling batch cooking in domestic settings. The inactivation parameters were obtained from Sym'Previus for *B*. *cereus* group III at 90°C and group IV at 100°C (**Table 1**). The cooking temperature was set at 95°C due to consumers' practice of constantly stirring and opening the lid of the pan. Heat inactivation was then computed per group following the heat inactivation model (**Eq. 8**):

$$D_{95\ BcIII} = D_{90BcIII}\ X\ 10^{(\frac{90-95}{ZBc\ III})}\ \text{and}\ D_{95\ BcIV} = D_{100BcIV}\ X\ 10^{(\frac{100-95}{ZBc\ IV})}$$

(**8**)

The probability of survival was calculated based on Nauta (2001) and was performed per *B*. *cereus* group following a similar formula:

$$P_{suriviving}^{BcIII} = 10^{(\frac{-tHT}{D95\ BcIII})}\ \text{and}\ P_{suriviving}^{BcIV} = 10^{(\frac{-tHT}{D95\ BcIV})}$$

(**9**)

The cooking times (tHT) differed between France, ranging from 25 to 35 min, while in Hungary it was 45–70 min. This difference indicates the cooked lentil dish most consumed in these two countries: lentil salad in France and lentil soup in Hungary. The variability surrounding the cooking times was described as a uniform distribution.

The number of surviving *B*. *cereus* spores was computed by multiplying the probability of survival per group by the initial contamination (**Eq. 10**). The variability surrounding the spore survivors was described using a Poisson distribution.

$$Q_{HT}^{BcIII} \sim Poisson\ (P_{suriviving}^{BcIV} x\ N_0^{BcIII})\ \text{and}\ Q_{HT}^{BcIV} \sim Poisson\ (P_{suriviving}^{BcIV} x\ N_0^{BcIV})$$

(**10**)

2.3.2.3. Module 3: Microbial germination of spores during the cooling of lentils and refrigerated storage of leftovers

The surviving spores can germinate and grow during the cooling and storage of lentil leftovers. This module was built based on the growth kinetics of *B*. *cereus* groups derived from Sym'Previus (Table 1) and conditions after cooking (e.g., cooling temperatures and time). In terms of the growth kinetics, the uncertainty surrounding lag times was expressed as uniformly distributed (Daelman et al., 2013; Laurent et al., 1999). The cooling temperature was assumed to be 19°C, and the cooling time was taken from the consumer survey (Yabré and Membré, 2022). The variability in the length of cooling time practiced by the consumer was described through an exponential distribution.

The microbial growth rate during cooling was computed following the gamma concept (Zwietering et al., 1996) (**Eq. 12**) with the Cardinal model and inflection for temperature from Rosso et al. (1993) **(Eq. 11**) and the optimal growth rate from Ellouze et al. (2021). Finally, the *B*. *cereus* concentration was computed following the exponential growth model (Buchanan et al., 1997) (**Eq. 13**) and subsequently converted to the number of microorganisms.

$$\gamma_T^{BcIII}\ or\ \gamma_T^{BcIV} = \begin{cases} T_{cooling} < T_{min},\ 0 \\ T_{cooling} > T_{max},\ 0 \\ \frac{(T_{cooling}-T_{min})^2\ (T_{cooling}-T_{max})}{(T_{opt}-T_{min})[(T_{opt}-T_{min})(T_{cooling}-T_{opt})-(T_{opt}-T_{max})(T_{opt}+T_{min}-2T_{cooling})]} \end{cases}$$

(**Eq.11**)

$$\mu_{cooling}^{BcIII\ or\ BcIV} = \mu_{opt}^{BcI\ III\ or\ Bc\ IV}\ x\ \gamma_T^{Bc\ III\ or\ Bc\ IV}$$

(**Eq.12**)

$$Q_{cooling}^{BcIII\ or\ Bc\ IV} = \begin{cases} Q_{HT}^{Bc\ III\ or\ Bc\ IV}\ if\ lag_{cooling} \geq t_{cooling} \\ Q_{HT}^{Bc\ IIIor\ Bc\ IV}\ x \exp\left[\mu_{cooling}^{BcIII\ or\ Bc\ IV}\ x\ (t_{cooling} - lag_{cooling})\right]\ if\ lag_{cooling} < t_{cooling} \end{cases}$$

(**Eq.13**)

2.3.2.4. Module 4: Microbial growth and storage conditions during refrigeration

Batch-cooked lentils are not consumed in one serving, and leftovers are kept in the refrigerator and consumed over several occasions. The differentiating factor between the two countries is the duration of storage, portion size, and number of portions per dish (**Table 1**). In terms of refrigeration temperature, the same values were applied for both countries (Roccato et al., 2017). The distinction between France and Hungary is the leftover storage time and frequency (Koppel et al., 2016; Yabré and Membré, 2022).

Microbial growth during this period was determined following a similar modelling approach as cooling, namely determining the growth rate during refrigeration with temperature in the fridge as the cardinal parameter (**Eq. 14**) in the gamma model (**Eq. 15**). This enabled the computation of the *B. cereus* concentration over time (**Eq. 16**) and was expressed as total *B. cereus* per serving of leftovers (**Eq. 17**).

$$\gamma_{fridge}^{BcIII}\ or\ \gamma_{fridge}^{BcIV} = \begin{cases} T_{fridge} < T_{min}^{BcIII\ or\ BcIV}, & 0 \\ T_{fridge} > T_{max}^{BcIII\ or\ BcIV}, & 0 \\ \dfrac{(T_{fridge} - T_{min}^{BcIII\ or\ BcIV})^2 (T_{fridge} - T_{max}^{BcIII\ or\ BcIV})}{(T_{opt} - T_{min})\left[(T_{opt} - T_{min}^{BcIII\ or\ BcIV})(T_{fridge} - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min}^{BcIII\ or\ BcIV} - 2T_{fridge})\right]} \end{cases}$$

(**14**)

$$\mu_{fridge}^{BcIII\ or\ BcIV} = \mu_{opt}^{BcIII\ or\ BcIV}\ x\ \gamma_{fridge}^{BcIII\ or\ BcIV}$$

(**15**)

$$Q_{fridge}^{BcIII}\ or\ Q_{fridge}^{BcIV} = \begin{cases} Q_{cooling}^{Bc\ III\ or\ Bc\ IV}\ if\ lag_{storage} \geq t_{storage} \\ Q_{cooling}^{Bc\ IIIor\ Bc\ IV}\ x \exp\left[\mu_{fridge}^{BcIII\ or\ BcIV} x\ (t_{storage} - lag_{storage})\right]\ if\ lag_{cooling} < t_{cooling} \end{cases}$$

(**16**)

$$Q_{serving}^{Bc\ total/Xh} = \frac{Q_{fridge}^{BcIII} + Q_{fridge}^{BcIV}}{No.servings\ dish}$$

(**17**)

2.3.2.5. Module 5: Risk estimation

The microbial risk from *B. cereus* was determined by first establishing the cut-off values and subsequently assessing the concentration reached per specific time (Xh) (**Eq. 18**). The cut-off values were taken from the literature, determining the concentration at which *B. cereus* causes foodborne illness (Anses, 2021).

$$Risk_{Xh}^{Bc} = Q_{serving}^{Bc\ total/Xh} > Bc_{cutoff} \times Portion\ size$$

(**18**)

Leftover lentils are either eaten cold or hot. During this iterative consumption of leftovers, it may be reheated or eaten cold, which can pose a microbial risk. The uncertainty of leftover times was

represented by a beta distribution (**Eq. 19**) based on a survey of lentil consumers (n = 161) storing cooked lentils (Yabré and Membré, 2022) and the prevalence per storage time (s) in France (**Table 1**). The prevalence for Hungary was appropriated from the values obtained in Italy, given its location (Koppel et al., 2016). The probability of serving conditions per specific time was computed (**Eq. 20**). Ultimately, the risk posed by *B*. *cereus* was computed for both the hot and cold servings (**Eq. 21**).

$$P^{Xh}_{Leftover\ time_{Fr\ or\ Hu}} \sim Beta\ (s+1, n-s+1) \quad \textbf{(19)}$$

$$P^{hot/cold}_{Xh} = P^{serve}_{hot\ or\ cold}\ x\ P^{Xh}_{Leftover\ time_{Fr\ or\ Hu}} \quad \textbf{(20)}$$

$$Risk^{Bc}_{cold\ or\ hot} = (P^{cold\ or\ hot}_{Xh} x R^{Bc}_{Xh} + \cdots + P^{cold\ or\ hot}_{nh} x R^{Bc}_{nh}) \quad \textbf{(21)}$$

2.3.3. Listeria monocytogenes exposure assessment model

2.3.3.1. Module 1: L. *monocytogenes contamination of batch-cooked lentils*

Listeria monocytogenes recontamination occurs during the storage of batch-cooked lentils in domestic refrigerators. The recontamination level was set to 1 bacterial count as the lowest possible contamination per dish from *L*. *monocytogenes* in the environment (Azevedo et al., 2005; Beumer et al., 1996). This was reflected per the portion size for France and Hungary (**Eq. 22**). The prevalence of contamination (s = 5) in domestic settings (n = 204) was described using a beta distribution (**Eq. 23**) (Beumer et al., 1996). The Bernoulli distribution was used to describe the variability of *L*. *monocytogenes* contamination (**Eq. 24**). The initial concentration of *L*. *monocytogenes* (log CFU/g) in cooked lentils was computed following **Eq. 25**.

$$N0_{Lm} = \frac{1}{(\chi_{portion}\ x\ Portion\ size_{Fr\ or\ Hu})} \quad \textbf{(22)}$$

$$Pr^{Lm}_{leftovers} = Beta\ (n+1, n-s+1) \quad \textbf{(23)}$$

$$Pb_{Lm} = Bernouilli\ (Pr^{Lm}_{leftovers}) \quad \textbf{(24)}$$

$$Q_{Lm} = N0_{Lm}\ x Bernouilli\ (Pb_{Lm}) \quad \textbf{(25)}$$

2.3.3.2. Module 2: Microbial growth kinetics during refrigerated storage of leftovers

The modelling approach for microbial growth is similar to the *B*. *cereus* model. The effect of refrigeration temperature on the growth rate was determined using the cardinal parameter following Eq. 11, which was adapted (**Eq. 26**) and incorporated into the gamma model (**Eq. 27)**. The growth parameters of *L*. *monocytogenes* were obtained from Anses (2020), and the growth rate from Combase (**Supplementary Table 2**). Subsequently, the microbial concentration per storage time (**Eq. 28**) was determined and expressed per serving (**Eq. 29**).

$$\gamma(T)_{LM} = \begin{cases} T_{fridge} > T_{max}\ ;\ 0 \\ T_{fridge} < T_{max}\ ;\ 0 \\ \dfrac{(T_{fridge} - T_{min})^2 (T_{fridge} - T_{max})}{(T_{opt} - T_{min})[(T_{opt} - T_{min})(T_{fridge} - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T_{fridge})]} \end{cases} \quad \textbf{(26)}$$

$$\mu_{Lm} = \mu_{optLm}\ \mathrm{x}\ \gamma(T)_{Lm} \quad \textbf{(27)}$$

$$Q_{Xh}^{Lm} = \begin{cases} Q_{Lm}\ if\ t_{storage} < lag_{storage} \\ Q_{Lm}\ x \exp[\mu_{Lm} x\ (t_{storage} - lag_{storage})]\ if\ t_{cooling} < lag_{cooling} \end{cases} \quad \textbf{(28)}$$

$$Q_{serving}^{Lm/Xh} = \frac{Q_{Xh}^{Lm}}{Number\ of\ servings\ per\ dish} \quad \textbf{(29)}$$

2.3.3.3. Module 3: Risk estimation from L. monocytogenes

The risk associated with lentil consumption was established by establishing the cut-off values for *Listeria monocytogenes* (**Table 1**). The lethal dose of virulent strains (LD_{50}) of *L. monocytogenes*, its concentration, and isolation in food samples from sporadic cases of listeriosis were determined (Pouillot et al., 2024) (**Eq. 30)**. Furthermore, the uncertainty associated with the LD_{50} was captured using a uniform distribution. The cut-off was expressed per mean portion size of lentils for France and Hungary (**Eq. 31).**

$$Pr.vir = (Pr_{less\_vir}\ x\ Q_{less\_vir}) x\ (Pr_{med_{vir}}\ x\ Q_{med_{vir}}) x\ (Pr_{high\_vir}\ x\ Q_{high\_vir}) \quad \textbf{(30)}$$

$$Lm_{cutoff} = 10^{Pr.vir}\ x\ \chi_{portion} \quad \textbf{(31)}$$

$$Risk_{Xh}^{Lm} = Q_{serving}^{Lm/Xh} > Lm_{cutoff} \quad \textbf{(32)}$$

$$Risk_{cold}^{Lm} = (P_{Xh}^{cold} x R_{Xh}^{Bc} + \cdots + P_{nh}^{cold} x R_{nh}^{Bc}) \quad \textbf{(33)}$$

2.3.3.4. Module 4: Total burden of lentil consumption from L. monocytogenes and B. cereus

The computation of risks from the two pathogens reflects the serving conditions and their related ability to pose food safety risks. For cold dishes, food safety risks from *B. cereus* and *L. monocytogenes* were combined (**Eq. 34**). The total risk for the lentil servings was calculated by combining all risks from subsequent portions of lentils (**Eq. 35**).

$$Risk_{cold}^{Lm-Bc} = 1 - \{(1 - P_{Bc}^{cold})\ x\ (1 - P_{Lm}^{cold})\} \quad \textbf{(34)}$$

$$Risk_{total} = 1 - \{(1 - Risk_{Bc}^{cold})\ x\ (1 - Risk_{Lm}^{cold}) * (1 - Risk_{Bc}^{hot})\} \quad \textbf{(35)}$$

2.3.4. Health burdens and daily adjusted life years (DALY) computation

The risk of listeriosis and *B. cereus* infection per cold and hot dishes combined was determined (**Eq. 36**) by computing the risk concerning the lentil consumers in the population. The daily adjusted life years (DALYs) associated with these increased cases were computed (**Eq. 37**).

$$No_{cold\ or\ hot}^{ill} = Risk_{cold}^{Lm-Bc}\ x\ Annual_{intake}\ x\ No.people\ eating\ lentils \quad (36)$$

$$DALY = No_{cold\ or\ hot}^{ill}\ x\ \frac{DALY/disease}{1000} \quad (37)$$

2.4. Model implementation

The exposure assessment models were developed in the R language (R version R x 64 3.5.2) and run on the Galaxy platform (usegalaxy.eu). The 2nd order Monte Carlo procedure was performed using the 'mc2d' package. The results are presented as the mean and its 95th confidence interval (uncertainty interval). Uncertainty reflects the lack of estimates for the prevalence of contamination and the percentage of consumer responses, for instance. Variability reflects the true heterogeneity, such as the number of people per household or the refrigerator temperature. A total of 1000 iterations were generated for the uncertainty dimension and 100,000 for the variability dimension.

3. Results

3.1. Microbial contamination and growth in home-cooked lentils

Microbial growth was determined from raw lentils until the leftovers were consumed (**Table 2**). In the first module, the mean initial *B*. *cereus* contamination levels and their uncertainty intervals were similar for cooked lentils, 2.99 $\log_{10}$ CFU/g (95% CI: 2.78; 3.13) in France and 3.49 (95% CI: 3.28; 3.63) in Hungary (**Table 2**). This concentration was reduced by cooking with slightly higher estimates in France at 1.77 $\log_{10}$ CFU/g (95% CI: 1.44–2.00) than in Hungary at 1.36 $\log_{10}$ CFU/g (95% CI: 0.98; 1.64). Nevertheless, these counts remained stable during a cooling period of 12 h at room temperature. During refrigerated storage, *B*. *cereus* grew after 48 h and reached 2 logs after 96 h in both France and Hungary. However, the counts reached in Hungary were lower than those in France at the end of the storage period.

The mean counts of *L*. *monocytogenes* were much lower throughout the refrigeration period. These means were −2.25 log CFU/g (95% CI: −2.64; −1.95) in France and −2.05 $\log_{10}$ CFU/g (95% CI: −2.45; −1.72) in Hungary at the beginning of the cold storage. After 96 h of refrigeration, *L*. *monocytogenes* counts were slightly higher but were similar in both countries: 0.48 $\log_{10}$CFU/g (95% CI: −0.20; 1.16) in France and 0.69 $\log_{10}$ CFU/g (95% CI: 0.07; 1.36) in Hungary.

Table 1. Inputs used in the microbial risk model

Description	Label	Stochastic data		Input value	Unit	Implementation in the model	References
		U	V				
Domestic handling conditions							
Cooking time of lentil salad (France) and soup (Hungary)	tHT		X	Uniform for France (min: 25, max: 35) and Hungary (min: 45, max: 70)	mins	Eq. 9	Recipe review online
Cooking temperatures in France and Hungary	-			95	°C	Eq. 8 fixed	Estimation assumed by researchers
Cooling temperature	$T_{cooling}$			19	°C	Eq. 11	(Insee, 2023)
Cooling time	$t_{cooling}$	X	X	Exponential distribution based on data and bootstrap	hrs	Eq. 13	(Yabré and Membré, 2022)
Refrigerator temperature in Northern Europe	T_{fridge}	X	X	Uniform distribution for mean: (min: 5.9°C, max: 6.5°C) and standard deviation (2.7–3.0°C) of refrigerators	°C	Eqs. 14 and 18	(Roccato et al., 2017)
Refrigeration time of leftovers in France	Xh			0, 24, 48, 72, 96	hrs	Eqs. 16 and 21 fixed	(Yabré and Membré, 2022)
Refrigeration time of leftovers in Hungary	Xh			0, 48, 96	hrs	Eqs. 16 and 21 fixed	Estimation assumed by researchers
Number of portions per dish	$No.\ portion\ per\ dish$			5 (Fr); 3 (Hu)		Eq. 17 fixed	Estimation assumed by researchers
Serving condition and prevalence	$P^{serve}_{hot\ or\ cold}$	X		Beta distribution built from a survey of lentil consumers (n = 29) declaring eating hot (s = 26) and cold (s = 3)	individuals	Eq. 20	(Yabré and Membré, 2022)
Consumption practices							
Prevalence of leftover storage times in France for batch-cooked lentils	$P^{Xh}_{Leftover\ time\ Fr}$	X		Beta distribution built from a survey of lentil consumers (n = 161) with prevalence of storage time (s) at 12 h (s = 1), 24 h (s = 24), 48 h (s = 49), 72 h (s = 58), 96 h (s = 29)	(%)	Eqs. 20 and 21	(Yabré and Membré, 2022)
Prevalence of leftover storage times in Hungary for batch-cooked lentils	$P^{Xh}_{Leftover\ time\ Hu}$	X		Beta distribution built from literature survey (n = 94) with leftover storage of 1 d (35.1%), 2–3 d (45.7%), 4–6 d (12.8%)	(%)	Eqs. 20 and 21	(Koppel et al., 2016)
Lentil consumption per day for France	$Portion\ size_{Fr}$	X	X	Gamma distribution based on data and bootstrap (France)	g/d	Eqs. 1, 18, and 22	INCA 3
Lentil consumption per day for Hungary	$Portion\ size_{Hu}$			Weibull distribution based on data and bootstrap (Hungary)			EU MENU
French dry lentil consumers				284	individuals		
Hungarian dry lentil consumers				87	individuals		
Bacillus cereus model							
Module 1: Initial microbial contamination							
Prevalence of *B. cereus* contaminated samples over tested legume samples	$N0$			Red lentils (6/8), yellow split beans (2/4), green split peas (1/3), kidney beans (2/3), and mung beans (0/3).			(Blakey and Priest, 1980)
Number of samples containing ≤ 2 log CFU/g^{-1}	$N^{Bc>2}$			2	No. of samples		(Blakey and Priest, 1980)
Number of samples containing ≥ 2 log CFU/g^{-1}	$N^{Bc<2}$			13	No. of samples		(Blakey and Priest, 1980)
Average portion size in France and Hungary *based on the mean value of the gamma	$\chi_{portion}$			39.3 g (France) and 72.9 g (Hungary)	g	Eq. 22	INCA 3 and EU Menu

Maximum concentration of *B. cereus* in legumes	C_{max}			45,000 CFU /g	No. of samples		
Prevalence of *B. cereus* group III strains	P_{GIII}^{Bc}	X		Uniform (20–80%);	%	Eq. 6	
Prevalence of *B. cereus* group IV strains	P_{GIV}^{Bc}	X		Uniform (1-P_{GIII}^{Bc});	%	Eq. 6	
Module 2: Inactivation during cooking							
D-value at 90 and 100°C for *B. cereus* groups III and IV	D_{90} *or* D_{100}			101.9 min (G III), 6.08 min (G IV)	min	Eq. 8	Sym'previus 2024
Z values for *B. cereus* Groups III and IV	$Z_{Bc\ III\ or\ IV}$			6.2°C (G III), 7.128°C (G IV)	°C	Eq. 8	Sym'previus 2024
Modules 3 and 4: Growth during cooling and storage							
Minimum temperature of growth	T_{min}			7.33°C (Group III), 7.44°C (Group IV)	°C	Eq. 11	Sym'previus 2024
Optimum temperature of growth	T_{opt}			39.06°C (Group III), 38.71 °C (Group IV)	°C	Eq. 11	Sym'previus 2024
Maximum temperature of growth	T_{max}			47.76°C (Group III), 47.44°C (Group IV)	°C	Eq. 11	Sym'previus 2024
Optimum growth rate	μ_{opt}			1.41 h^{-1} (Groups III and IV)	h^{-1}	Eq. 12	(Ellouze et al., 2021)
Lag time during cooling (germination)		X		Uniform (min = 6, max = 8)	h	Eq. 13	(Laurent et al., 1999)
Lag time during storage		X	X	Uniform (min = 12, max = 72)	h	Eq. 13	(Daelman et al., 2013)
Module 5: Risk estimation							
B. cereus concentration at toxin production	Bc_{cutoff}			10^5			(Anses, 2021)
Cut-off value		X	X	(*B. cereus* toxin prod × mean portion size)	L		
Listeria monocytogenes model							
Module 1: Initial microbial contamination							
L. monocytogenes in the environment		X		1 cell			
Prevalence of houses positive with *L. monocytogenes*	$Pr_{leftovers}^{Lm}$	X	X	5/204 houses (2.45 %)		Eq. 23	(Azevedo et al., 2005; Beumer et al., 1996)
Module 2: Growth during storage time							
Minimum temperature of growth	T_{min}			−2	°C	Eq. 26	(Anses, 2020)
Optimum temperature of growth	T_{opt}			45	°C	Eq. 26	
Maximum temperature of growth	T_{max}			33.5	°C	Eq. 26	
Optimum growth rate from the literature	μ_{opt}	X		Normal distribution (mean: 0.3217, SD: 0.02084)	$h^{-1/2}$	Eq. 27	(see Supplementary Table 2)
Module 3: Risk estimation							
LD_{50} of the virulent strains	Q_{less_vir}	X	X	Uniform distribution (6.80–9.70 log CFU), Less virulent strain		Eq. 30	(Pouillot et al., 2024)
	$Q_{med_{vir}}$	X	X	Uniform distribution (4.49–6.23 log CFU) Virulent strains		Eq. 30	
	Q_{high_vir}	X	X	Uniform distribution (2.57–3.67 log CFU) More virulent strains		Eq. 30	
Strain prevalence in food products from reported sporadic cases in the EU from 254 samples				Number of samples = 254: 122.9 (42%) low virulence, 102.3 (35%) moderate virulence, 64.9 (22%) high virulence		Eq. 30	(Pouillot et al., 2024)

Table 2. *Bacillus cereus* and *Listeria monocytogenes* concentration and risk per portion in lentil dishes and leftovers

Country	Microorganism	Storage time	Concentration (log_{10}CFU/g)	Risk per portion
			Mean [2.5th;97.5th]	
France	*B. cereus*	0	2.99 [2.78; 3.13]	
		After cooking	1.77 [1.44; 2.00]	
		After cooling	1.83 [1.50; 2.08]	
		24 h refrigeration	1.83 [1.50; 2.08]	0.00 [0.00; 0.00]
		48 h refrigeration	1.87 [1.53; 2.17]	0.00 [0.00; 0.00]
		72 h refrigeration	2.02 [1.58; 2.86]	0.00 [0.00; 0.00]
		96 h refrigeration	2.52 [1.75; 4.12]	0.00 [0.00; 7.00×10^{-4}]
	L. monocytogenes	0	−2.25 [−2.64; −1.95]	0.00 [0.00; 0.00]
		24 h refrigeration	−1.82 [−2.22; −1.51]	0.00 [0.00; 0.00]
		48 h refrigeration	−1.24 [−1.67; −0.88]	0.00 [0.00; 0.00]
		72 h refrigeration	−0.47 [−0.99; 0.03]	0.00 [0.00; 0.00]
		96 h refrigeration	0.48 [−0.20; 1.16]	0.00 [0.00; 0.00]
Hungary	*B. cereus*	0	3.49 [3.28; 3.63]	
		After cooking	1.36 [0.98; 1.64]	
		After cooling	1.37 [1.00; 1.65]	
		48 h refrigeration	1.41 [1.03; 1.72]	0.00 [0.00; 0.00]
		96 h refrigeration	2.03 [1.24; 3.56]	0.00 [0.00; 9.02×10^{-5}]
	L. monocytogenes	0	−2.05 [−2.45; −1.72]	
		48 h refrigeration	−1.04 [−1.43; −0.66]	0.00 [0.00; 0.00]
		96 h refrigeration	0.69 [0.07; 1.36]	0.00 [0.00; 0.00]

The microbial counts per serving and their cumulative distribution are shown with the cut-off values in France (**Figure 1**) and Hungary (**Figure 2**). Low microbial count estimates were observed in most of the serving portions. In particular, the plot of *B. cereus* (**Figure 1**) showed that the counts estimated in France varied between 0 and 3 log_{10} CFU/g in most of the lentil portions (approximately 60–80%) over the storage time (0–96 h). A slight difference in the counts was observed in Hungary, with variability of 0–2 log_{10} CFU/g in most of the lentil portions (approximately 60–80%). The opposite was observed for *L. monocytogenes*, where the variability in the counts and the uncertainty around it were very small. Nevertheless, very few serving instances exceeded the cut-off values for *B. cereus* and all *L. monocytogenes* strains (low, moderate, and high virulence strains).

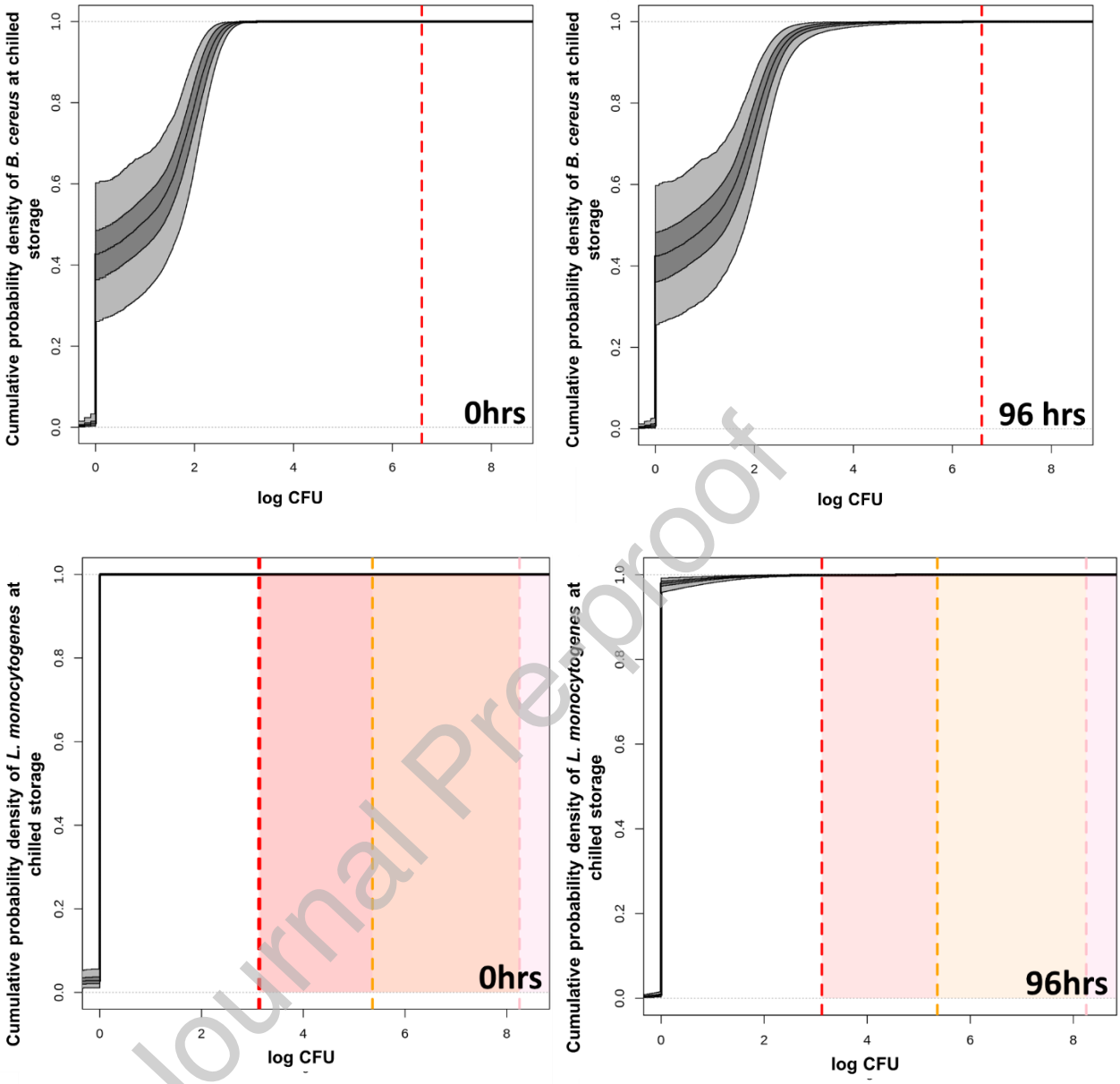

Figure 1. The cumulative density of *Bacillus cereus* (upper panel) and *Listeria monocytogenes* (lower panel) in batch-cooked lentils in France at 0 and 96 h of storage. The dark grey region of the cumulative density curve corresponds to the 25th and 75th percentiles of the uncertainty, and the light grey region corresponds to the 95% uncertainty intervals. The figures for *B*. *cereus* show the single cut-off values. The *L*. *monocytogenes* figures show three cut-off values for low (yellow line), moderate (orange line), and high (red line) virulence strains.

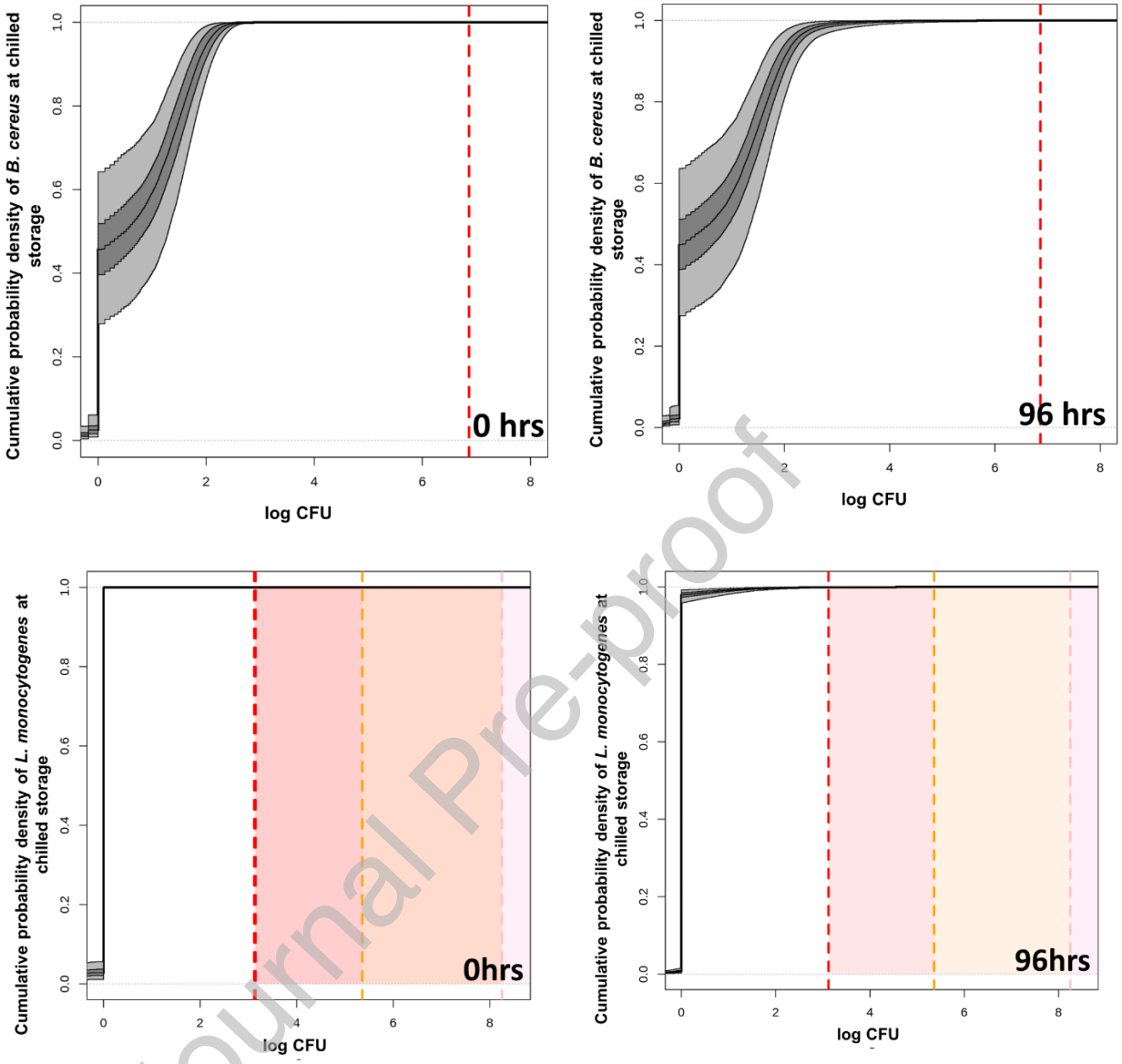

Figure 2. The cumulative density of *Bacillus cereus* (upper panel) and *Listeria monocytogenes* (lower panel) in batch-cooked lentils in Hungary at 0 and 96 h of storage. The dark grey region of the cumulative density curve corresponds to the 25th and 75th percentiles of the uncertainty, and the light grey area corresponds to the 95% uncertainty intervals. The figures for *B*. *cereus* show the single cut-off values. The *L*. *monocytogenes* figures show three cut-off values for low (yellow line), moderate (orange line), and high (red line) virulence strains

3.2. Risk estimates per portion from home-cooked lentils and leftovers

The mean risk estimates per portion were determined over the storage time (**Table 2**). For *L*. *monocytogenes*, these estimates were null across the chilled storage period. In contrast, a non-zero risk per portion was estimated for *B*. *cereus* after 96 h of refrigeration. This can be observed within the upper uncertainty interval (CI) of the mean estimates at 7.00×10^{-4}. The risk per portion at the upper CI was slightly higher for France than for Hungary, which was at 9.02×10^{-5}.

The overall risk associated with the lentil portions and their respective serving conditions (e.g., hot or cold) was determined (**Table 3**). *Bacillus cereus* was accounted for in both cold and hot served portions, while *L*. *monocytogenes* was only considered in cold servings. Very low risks were observed under both serving conditions, but with non-zero estimates in the upper CI. The hot-served portions were estimated to have slightly higher risks, with France having higher upper CI estimates at 1.05×10^{-4} than Hungary at 2.53×10^{-5}. The same trend between countries was observed for cold servings of lentil dishes. In terms of *L*. *monocytogenes*, zero risk was estimated in both countries for the mean and CI values.

The combined risk for all cold servings of lentil dishes reflects the previous zero mean values, except the upper-bound CI estimates. Between-country comparisons of total risks reflect the same trend, with France having higher risk estimates. The next step is to contextualise the risk estimated with the additional total burden of disease at the population level.

Table 3. Estimated risk per portion of lentil dishes in France and Hungary.

	Serving conditions	Risk [Mean, 95% CI]	
		France	**Hungary**
B. *cereus*	Hot served	0.00 [0.00; 1.05×10^{-4}]	0.00 [0.00; 2.53×10^{-5}]
	Cold-served	0.00 [0.00; 1.70×10^{-5}]	0.00 [0.00; 3.82×10^{-6}]
L. *monocytogenes*	Cold-served	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]
Risk from all cold servings		0.00 [0.00; 1.70×10^{-5}]	0.00 [0.00; 3.82×10^{-6}]
Total risk from cold and hot servings		0.00 [0.00; 1.16×10^{-4}]	0.00 [0.00; 2.84×10^{-5}]

3.3. Health burden from *L*. *monocytogenes* and *B*. *cereus* contamination

The estimated risks were computed as the attributable foodborne illness cases in the entire population or the number of illnesses per year (**Table 4**). The results have demonstrated that the mean estimates were zero for the number of people getting sick due to cold or hot dishes. However, the non-zero case estimates were observed within the upper bounds of the CI estimates.

The pathogen driving the cases was *B*. *cereus*, with *L*. *monocytogenes* as a zero-projected case. The hot lentil serving portions posed a potentially higher risk than cold dishes, with upper-bound CI mean estimates of 27,101 cases in France and 493 cases in Hungary. In terms of the overall number of illnesses, these were higher for France (29,925 cases) than in Hungary (554 cases).

These cases were expressed in DALYs by utilising the data on the average intakes and annual lentil consumption of the whole population of these two countries. This computation determined the additional health burden associated with these projected cases. Zero mean estimates were also observed with the upper CIs resulting in non-zero DALYs. Higher additional health burden were attributed to the hot servings than to the cold portions.

Table 4. Estimated burden of disease for the French and Hungarian population associated with lentil-based dishes.

	France	**Hungary**
Burden estimates		
Lentil consumers and population statistics		
Average portion size (g)	39	73
The annual number of lentil intakes	94	122
Number in the population	68,000,000	9,590,000
% Consumers of dry lentils	4%	5.50%
Number of people eating dry lentils	2,720,000	527,840
Health impact per year	**Mean [2.5th; 97.5th]**	
No of illnesses due to the cold serving *(B. cereus)*	0.00 [0.00; 4,406]	0.00 [0.00; 75]
No of illnesses due to the cold serving *(L. monocytogenes)*	-	-
No of illnesses due to the hot serving *(B. cereus)*	0.00 [0.00; 27,101]	0.00 [0.00; 493]
Total number of illnesses	0.00 [0.00; 29,925]	0.00 [0.00; 554]
Total number of illnesses per 100,000	0.00 [0.00; 44]	0.00 [0.00; 5.8]
DALYs		
DALYs of illnesses due to the cold serving *(B. cereus)*	0.00 [0.00; 10.13]	0.00 [0.00; 0.17]
DALYs of illness due to the cold serving *(L. monocytogenes)*	-	-

DALYs of illnesses due to the hot serving *(B. cereus)*	0.00 [0.00; 62.33]	0.00 [0.00; 1.13]

4. Discussion

The results of this study indicate that the risk associated with lentil consumption is low in comparison with other dietary sources, such as broiler and red meat, raw vegetables, and dairy products (EFSA, 2023). These were implicated in hospitalised foodborne cases of listeriosis, salmonellosis, and STEC infections. Nevertheless, the selection of lentils was due to its promotion among the non-meat protein alternatives, given its multiple positive impacts in terms of agriculture and nutritional benefits (Iqbal et al., 2006; Romano et al., 2021; Tidåker et al., 2021). The focus on domestic settings lies in their vulnerability to microbial risk compared to other product forms (e.g., direct consumption of canned food). The results indicate that there is a very small risk, but not zero, which, if considered at a population level, can still have an impact.

The current study determined a very low microbial risk with lentil consumption in France and Hungary. The findings of this study point to an impact when translated at a regional or national level, particularly in France and Hungary, where legume consumption is encouraged as part of the "healthy plate" (i.e., French National Nutrition and Health Program PNNS, 2019 and Hungarian nutritional recommendation Okostányér, 2021). These may influence people to consume more, including the positive perception associated with it. Therefore, regulators, particularly those who promote dietary shifts, must employ QMRA and other preliminary assessments. These estimates may change in other legume products, which are outside the scope of this study and the model developed.

The non-zero estimates highlight the need to incorporate variability and uncertainty surrounding the inputs. These estimates can still be further refined by incorporating better data on domestic practices. Overall, it can be said that good cooking and domestic practices are currently being observed. Nevertheless, if people are pushing the storage time (e.g., 6 days), the risk estimates will change. From this, we can calculate different scenarios focusing on domestic practice changes. Other bacteria (e.g., spoilage bacteria) might come into play. Despite this change, the current model and associated modelling strategy can still be used with modification of the input variables (e.g., growth rate).

The initial contamination and duration of refrigeration influenced the microbial concentration of leftovers over time. The initial counts were based on the literature (Beumer et al., 1996; Blakey and Priest, 1980), but a more recent study for these countries was not available for both bacteria. This indicates the need for more updated data on dried lentils, as consumption is being promoted. The change in the prevalence and levels of *B. cereus* spores can increase or reduce the estimated risks to the population. The probability associated with the prevalence of *B. cereus* in these packages was described by a beta distribution. This is a continuous distribution commonly used to describe the presence of *B. cereus* in packaged food products (Kwon et al., 2020; Wang et al., 2023). The initial concentration was described using the Bernoulli distribution. These counts were reduced by heating and significantly reducing microbial risks. *Bacillus cereus* grew during refrigeration, which partially undoes the effect of cooking. This is similar to the study of Kobayashi et al. (2021), who demonstrated the ability of *B. cereus* strains to grow after 4–10 days at 6–10°C. This study reports on psychrotrophic and non-psychrotrophic strains in nutrient broth, and growth was estimated using lab medium after 4–28 days of incubation at constant temperatures (4, 6, 8, and 10°C) from 5 log spores/mL to visible turbidity.

The presence of *L. monocytogenes* in leftovers suggests recontamination events after batch cooking. The low microbial counts reflect the low prevalence of *L. monocytogenes* in domestic refrigerators. In addition, if present, these were found in low levels (0–1 log CFU) (Beumer et al., 1996). Nevertheless, the prevalence used in this study is similar in Portugal (3.9%) and Greece (4.5%) (Azevedo et al., 2005; Sergelidis et al., 1997). Recontamination events have been the main driving cause of *L. monocytogenes* in foods and are linked to foodborne outbreaks in home and food manufacturing facilities (Møretrø et al., 2024; Zhang et al., 2022). The results demonstrate that current lentil cooking and post-cooking practices in France and Hungary have not significantly introduced recontamination. A more complex post-cooking scenario (e.g., addition of fresh ingredients in batch-cooked lentils) and cross-contamination can impact these estimates (Possas et al., 2017). In addition, future scenarios can influence the food safety of lentils. Several studies have demonstrated that room temperatures can influence the microbial risk. This was demonstrated by the microbial spoilage risk of fruit juice (Kakagianni et al., 2016), evaporated canned milk (Kakagianni and Koutsoumanis, 2018; Koutsoumanis et al., 2022), and plant-based alternatives (Misiou et al., 2023). These results highlight

that existing and projected temperatures during storage can impact spoilage due to the consequent change in the microbial concentration. Therefore, caution should be applied in the future, as there is a need for a more systematic evaluation of climate change in microbial risk assessment. The refrigeration temperatures used in this study are based on actual refrigerator temperatures for countries located in Western Europe. Refrigeration temperatures vary depending on location (Roccato et al., 2017). These changes, whether due to location or climate change, may affect food safety risks that can come from lentil consumption. These values must change accordingly if the current model is to be adopted in other countries, which may affect food safety risks. This has been observed in a similar study where concentrated milk has a higher spoilage probability due to hotter room temperatures and higher refrigeration temperatures (Misiou et al., 2023).

The microbial safety risk of lentil portions was determined using cut-off values, which, if reached, will result in foodborne illness. The cut-off values were derived from scientific literature detailing the microbial concentration levels linked to foodborne illness for *B*. *cereus* and *L*. *monocytogenes*. The *B*. *cereus* cut-off values derived for this study are based on 10^5 CFU/g, which assumes that at this level, it can cause foodborne illness (Anses, 2021). This was the best available data given that a dose–response study with LD_{50} was not available for *B*. *cereus* cells or cereulide toxins (Rouzeau-Szynalski et al., 2020). The current model measures the risk from cold and hot dishes, reflecting both foodborne intoxication of *B*. *cereus* due to cereulide toxin and infection from vegetative cells. By ingesting toxins, we only evaluate emetic symptoms, and these symptoms are declared at 10^5 CFU/g of food. However, sometimes, food contaminated with 10^3 is sufficient to cause illness, and other times, more than 10^8 is needed (Anses, 2021).

The prevalence of *B*. *cereus* strains in different food samples was not available. Therefore, it was assumed that *B*. *cereus* belonged to group III or IV, which are virulent strains according to the literature (Guinebretière et al., 2010; Mombert et al., 2024). *Listeria monocytogenes* is well-studied with existing LD_{50}, and the prevalence of virulent strains in food samples enhanced the estimates (Pouillot et al., 2024).

The low-risk estimates of foodborne illness were estimated for all lentil dishes except for the upper-bound CI estimates. These show the uncertainty in the estimates, with large variability in the results. These non-zero estimates of risk translate into a high number of occurrences of foodborne illnesses per year at the population level. This finding demonstrates the importance of probabilistic risk assessment in capturing the uncertainty across the model. Furthermore, if these estimates were translated at the population level, it would result in projected cases of foodborne illnesses. Such information can help in estimating the additional burden of lentil consumption on the population. This bridges the gap between domestic practices and food safety in the supply chain and the public health impact. Furthermore, the results can guide public health officials in evaluating any future lentil consumption promotion policies. When comparing serving conditions, a high risk associated with hot dishes can be linked to a higher frequency of consumption. This can be linked with the trend in France and Hungary to reheat batch-cooked lentils.

The current study points to domestic practices (i.e., cooking, cooling, reheating, and refrigeration) and hygiene at home as the main drivers of microbial risks. These highlight the role of heating in reducing the initial microbial load. The cooling at room temperature for 12 h in this situation (i.e., low contamination level, low room temperature, high heat treatment) was not shown to pose microbial risks. Nevertheless, leftovers must be kept at a temperature lower than 6°C and consumed within 72 h. In France, this decrease in temperature must be achieved within 2 h. It was also demonstrated that food safety practices play a role, such as maintaining the cold temperature for cooked meals and proper cooking of foods. The implications of domestic practices go beyond the current scenario to other legume-based foods prepared in mixed dishes (e.g., legume with sausages). The existing risk associated with domestic preparation and storage practices will drive the risk in full or partial plant-based diets. This is expected to rise as the intake of legumes increases. Therefore, current domestic practices must be considered as lentil consumption is promoted (Ministère des Solidarités et de la Santé, 2019; National Institute of Pharmacy and Nutrition, 2021; PNNS, 2019; Okostányér, 2021).

These results can also guide institutional catering establishments that employ large-scale batch-cooking practices and storage. Lentil consumption is also being pushed in these settings as part of an effort to consume alternative proteins in both France and Hungary (Magrini et al., 2021; Nagy et al., 2021).

The use of microbial risk assessment can also be done in other contexts, such as mixed food settings and with other protein alternatives. Separate microbial risk assessments must be performed with respect to its hazards, processing, and associated domestic practices. Nevertheless, these future assessments may be informed by the current study through the inputs used and the modelling framework applied. Additionally, the current study can be combined with these future assessments of other protein alternatives to have a more complete picture of the risk of protein alternatives and their impact on the overall diet of the population. Therefore, updated data are needed to determine the prevalence of virulent *B*. *cereus* strains and microbial concentration levels in imported or locally produced lentils. An updated survey on *L*. *monocytogenes* in domestic settings and refrigerators from these two countries is needed. The consumer data used (INCA 3) is the most comprehensive data available for the French population in terms of mean consumption. Its new version is still being prepared by the Santé publique France and Anses, which is called the Albane survey (https://www.enquete-albane.fr/). The current modelling approach can enhance future estimates of risks and additional health burdens to the population.

Food consumption trends, particularly those of a plant-based diets, are on the rise due to their nutritional benefits and low environmental impact. These can also be linked to their positive consumer perception (Spendrup and Hovmalm, 2022). However, despite these plant-based foods being linked to several chemical risks (e.g., heavy metals and mycotoxins) (Lin et al., 2023). In lentils, the heavy metal cadmium, which is introduced in the field due to the use of chemical fertilisers, has been found in France (Plateforme de Surveillance de la Chaine Alimentaire, 2023) and other countries (Six and Smolders, 2014; Zhang et al., 2020). Therefore, chemical risk assessment should be performed in future studies of lentil consumption and the overall lentil supply chain. Several studies have also employed risk–benefit analysis in determining the chemical risk and nutritional benefit of substituting red meat with pulses (Fabricius et al., 2021).

In terms of microbial risk, the reduction of meat consumption can lead to the reduction of foodborne illnesses linked to meat consumption (e.g., *Salmonella*, *Escherichia coli*, and *Campylobacter*) (Warmate and Onarinde, 2023). This can also reduce the introduction of meat in domestic settings, as microbes are usually cross-contaminated from meat and spread across kitchen surfaces and utensils (van der Vossen-Wijmenga et al., 2025). Overall, these highlight the necessity of including the assessment of microbiological risk before recommending new diets.

The current study contributes to this juncture but from a microbial food safety perspective. This research highlights the added value of QMRA in determining the health burden that may arise from any unintended and implicit microbial risks. First, it estimates the microbial risk due to the consumption of one food product, and then, it promotes good consumer practices that can help minimise risks (if possible).

In addition to these possible tools, a more nuanced and holistic view of food policy on dietary shifts must be considered. Steering consumer consumption is not easy, as it must be based on individual choices. Ultimately, policies that aim for “dietary shifts” must be an inclusive and multi-stakeholder approach that respects consumer rights in all of its aspects.

Indeed, microbial risk, chemical risk, and nutritional benefit are not the only risks that may arise from the food supply chain. As mentioned in the introduction, environmental impact must also be considered, and a win–win solution must be attained. A recent publication highlights the different tools and frameworks that can be used for holistic risk assessments (Országh et al., 2024). This paper is a part of a bigger study incorporating other dimensions.

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Credit authorship contribution statement

Rodney J. Feliciano: Conceptualization, Data curation, Formal analysis, Software, Writing – original draft, review and editing, Visualization.

Jeanne-Marie Membré: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Software, Writing – review & editing.

Louis Delaunay: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – review & editing.

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.

Graphical Abstract