Comprehensive REVIEWS in Food Science and Food Safety

Risk Assessment and Management of *Listeria Monocytogenes* in Ready-to-Eat Lettuce Salads

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Abstract: Foodborne diseases constitute a major concern in societies, and their causes are aimed to be identified and minimized. Only in the last few years, this is encouraged by the application of risk assessment, management, and communication. This work presents a probabilistic quantitative microbiological risk assessment and management of *Listeria monocytogenes* in ready-to-eat lettuce salads in Spain. For risk assessment, a guideline provided by Codex Alimentarius was followed. Food chain was modeled from processing of raw material at the factory up to consumption. Different assumptions were made to describe the variables of the model by probability distributions or mathematical models. Monte Carlo simulations of the model were run to estimate the number of cases in low-risk and high-risk populations. Although results deviated from the number of cases observed in Spain, given an ideal situation of 100% compliance of the microbiological criterion ≤100 cfu/g throughout the shelf-life of the product, the resulting number of cases was near the real situation. From the 4 risk management measures simulated, the injection of a mixture of gases into packages at manufacture (CO_2 about 5.5%, O_2 about 3%, and N_2 for the balance) was the most effective in reducing the number of cases, followed by 4 d of storage at home and prevention of high-risk consumers from consumption of ready-to-eat lettuce salads. More research and cooperation between different stakeholder organizations are needed in order to progressively improve the model. With this work, a breakthrough has been made with regards to risk assessment and management procedures and implementation.

Introduction

Diseases caused by foodborne pathogens constitute a worldwide public health problem and preventing them is a major goal of societies. Not only public health status is altered when foodborne diseases are reported, but also economic impacts in the population such as the costs derived from product recall, loss of customers, and potential costs of compensation are important issues to be considered (Garrido and others 2010). Assessment of the risk posed by potential hazards is necessary for governments in order to select risk management (RM) measures for food trade at national and international levels, avoiding risks to the population (Van Schothorst 1997). Also, food production, food supply, and household preparation of food are stages where stakeholders can profit from risk assessment results.

Listeria monocytogenes has been implicated in large well-documented foodborne outbreaks and sporadic cases in ready-to-eat (RTE) foods (Schlech and others 1983; Ho and others 1986; Bille 1990; McLauchlin and others 1991; Goulet and others 1995; Autio and others 1999; Aureli and others 2000). Consistently, this microorganism has been isolated from a wide range of raw and RTE meats, poultry, dairy products, and vegetables as well as from various food processing environments (Genigeorgis and others 1990; Jeong and Frank 1994; Arnold and Coble 1995; De Simón and Ferrer 1998; Nørrung and others 1999; Guerra and others 2001; Miettinen and others 2001; Gombas and others 2003; Vitas and others 2004; Flores and others 2004; Gudbjornsdottir and others 2004; Thevenot and others 2005). The ubiquitous nature and great persistence of this pathogen in different environments make its eradication very difficult. Despite the low burden of listeriosis observed in the European Union, the assessment of the risk posed by the pathogen is of high relevance, mainly due to (1) the high mortality rate of the disease (20 to 40%) (McLauchlin 1993; Rocourt 1994) and (2) the wide spread of the pathogen in foods and the environment.

RTE salads constitute an expanding food commodity nowadays. While the safety of RTE salads has been extensively studied (Nguyen-The and Carlin 1994; Francis and others 1999; Sapers and others 2006), little attention has been paid to impact evaluations of the incidence of *L. monocytogenes* in RTE salads, namely, risk assessment. In 2003, HHS-FDA and USDA-FSIS (2003) carried out a risk assessment of listeriosis for 23 categories of RTE products, including vegetables; a relatively low risk (<1 case/year) was predicted for vegetables. These authors reported that due to the high uncertainty caused by the diversity of the products considered, a need was suggested for additional investigations and the subdivision of the vegetables category into several different groups. Recently, Franz and others (2010) have published a risk assessment for *Escherichia coli* O157:H7, *Salmonella,* and

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L. monocytogenes in leafy green vegetables consumed at salad bars, focusing on the pathogen growth in the supply chain and the restaurant.

The implication of vegetables in cases of listeriosis (Schlech and others 1983; Ho and others 1986; Allerberger and Guggenbichler 1989), together with the serious consequences of the disease and the general concern about the presence of *L. monocytogenes* in food, particularly foods in which the pathogen may grow, like RTE leafy green salads (Carrasco and others 2008), make the risk assessment and management of *L. monocytogenes* in RTE lettuce salads be worthwhile. Szabo and others (2003) reported the same justification in their work on assessment of control measures to achieve a Food Safety Objective <100 cfu/g of *L. monocytogenes* in fresh pre-cut iceberg lettuce; however, they did not provide a formal risk assessment as introduced by Codex Alimentarius (1999). Besides, the increase in the risk of listeriosis that may be occurring as a consequence of the social changes in Spain in the last few decades deserves special attention and assessment. The most important changes are the increase of the elderly population (high-risk population) and the scarcity of time for preparing meals at home, which favor the purchase of RTE foods.

The objectives of this review are: (1) to demonstrate a modeling procedure to estimate the risk of listeriosis in the Spanish population by consumption of RTE lettuce salads following the recommendations of Codex Alimentarius (1999); (2) to detect and rank the factors influencing the risk of listeriosis (sensitivity analysis); and in the light of the previous results, (3) to estimate the impact of implementation of different RM measures to reduce the burden of listeriosis.

Risk Assessment Methodology and Data Sources

Framework and working tools

This work conducts a probabilistic quantitative microbiological risk assessment (QMRA) of *L. monocytogenes* in RTE lettuce salads in Spain, following the guideline provided by Codex Alimentarius (1999). The QMRA covers all steps along the food chain up to consumption. Figure 1 shows the general scheme of the QMRA model.

The QMRA model was built in an Excel spreadsheet and simulated by using @Risk Professional© software V. 4.5 (Palisade, Newfield state, N.Y., U.S.A.). Sensitivity analysis of the model allowed for selection of appropriate RM measures, which were implemented by modification of the QMRA model above.

Hazard identification

Codex Alimentarius (1999) defined *hazard identification* as "the identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods."

L. monocytogenes is a foodborne pathogen for humans and animals. It causes listeriosis in humans, with a variety of symptoms including mild diarrhea, meningitis, and septicemia (Farber and Peterkin 1991). *L. monocytogenes* has been found in a wide variety of food commodities, and evidence suggests that most exposure is foodborne. Raw and RTE vegetables have been reported to be contaminated by *L. monocytogenes* (Sizmur and Walker 1988; McLauchlin and Gilbert 1990; De Simón and others 1992; Arnold and Coble 1995; García-Gimeno and others 1996; Francis and others 1999; Nørrung and others 1999; Szabo and others 2000; Guerra and others 2001; Sagoo and others 2003; Loncarevic and others 2005). Beside this, several listeriosis outbreaks worldwide have been attributed to consumption of RTE vegetables (Schlech and others 1983; Ho and others 1986; Fain 1996; Salamina and others 1996). This fact points out that *L. monocytogenes*, if present on raw vegetables, may not be fully eliminated by commercial disinfection procedures (such as sanitary washing) applied in the manufacture of RTE vegetables. Although listeriosis is not frequent, generally at somewhere between 2 and 7 cases per million population, between 20% and 40% of the cases are fatal (McLauchlin 1993; Rocourt 1994). Major risk factors for acquiring listeriosis are immunosuppression, old age, and pregnancy. In Spain, 88 cases of listeriosis were diagnosed in 2008, representing an incidence of 0.2 cases per 100000 population. In previous years, the number of reported cases was 81, 78, 68, and 100 in 2007, 2006, 2005, and 2004, respectively (EFSA 2010). These numbers confirm the fact that listeriosis is a relatively rare disease compared to other common foodborne illnesses such as salmonellosis.

Despite the fact that listeriosis is associated with only a few virulent strains, all strains of *L. monocytogenes* were assumed as pathogenic to humans in this work. In this sense, McLauchlin (1997) stated that "in the interests of public safety and for considerations for public health purposes, all *L. monocytogenes*, including those recovered from food, should be regarded as potentially pathogenic."

Exposure assessment

Exposure assessment was defined by Codex Alimentarius (1999) as the "qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food, as well as exposures from other sources if relevant." In this study, only food was considered as a means of transmission of *L. monocytogenes*. A realistic representation of the exposure of the target population (Spain in this work) to *L. monocytogenes* should be provided. For this, it was necessary to gather data regarding (1) population, (2) consumption, and (3) *L. monocytogenes* status (prevalence and concentration) in the food at the time of consumption. These data were adequately combined following a mathematical and statistical approach.

Population. The present QMRA was based on the Spanish population. The Statistical National Institute of Spain (INE 2010) reported a population size of 46745807 inhabitants in 2009. However, assuming that children under 2-y-old do not consume RTE salads, the population size submitted to analysis was 45282830. A distinction between low-risk and high-risk populations was made with the aim of a more accurate assessment of the risk. For this purpose, the fractions reported by FAO/WHO (2004b) of the total population corresponding to high-risk individuals were applied to the Spanish population. FAO/WHO (2004b) stated that, among adults, high-risk groups should include adults over 65-y-old, pregnant women, and individuals with an impaired immune systems and certain medical conditions, such as cancer and recent organ transplantation.

Consumption patterns of RTE lettuce salads. The frequencies of consumption of RTE lettuce salads obtained in previous work (Carrasco and others 2007) were assumed for the Spanish population.

Such frequencies (*occasionally* = 45.7%, *once or twice a month* = 29.6%, *once or twice a week* = 17.3%, and *more than twice a week* = 7.4%) were modeled to obtain a distribution of the number of servings consumed per individual and year (S). Three-step modeling was undertaken to estimate S. First, each of the 4 frequencies was described by appropriate probability distributions. Second, all discrete sampled values from such distributions were weighted according to the frequency percentages above. Last, some distributions were fitted to the overall weighted values

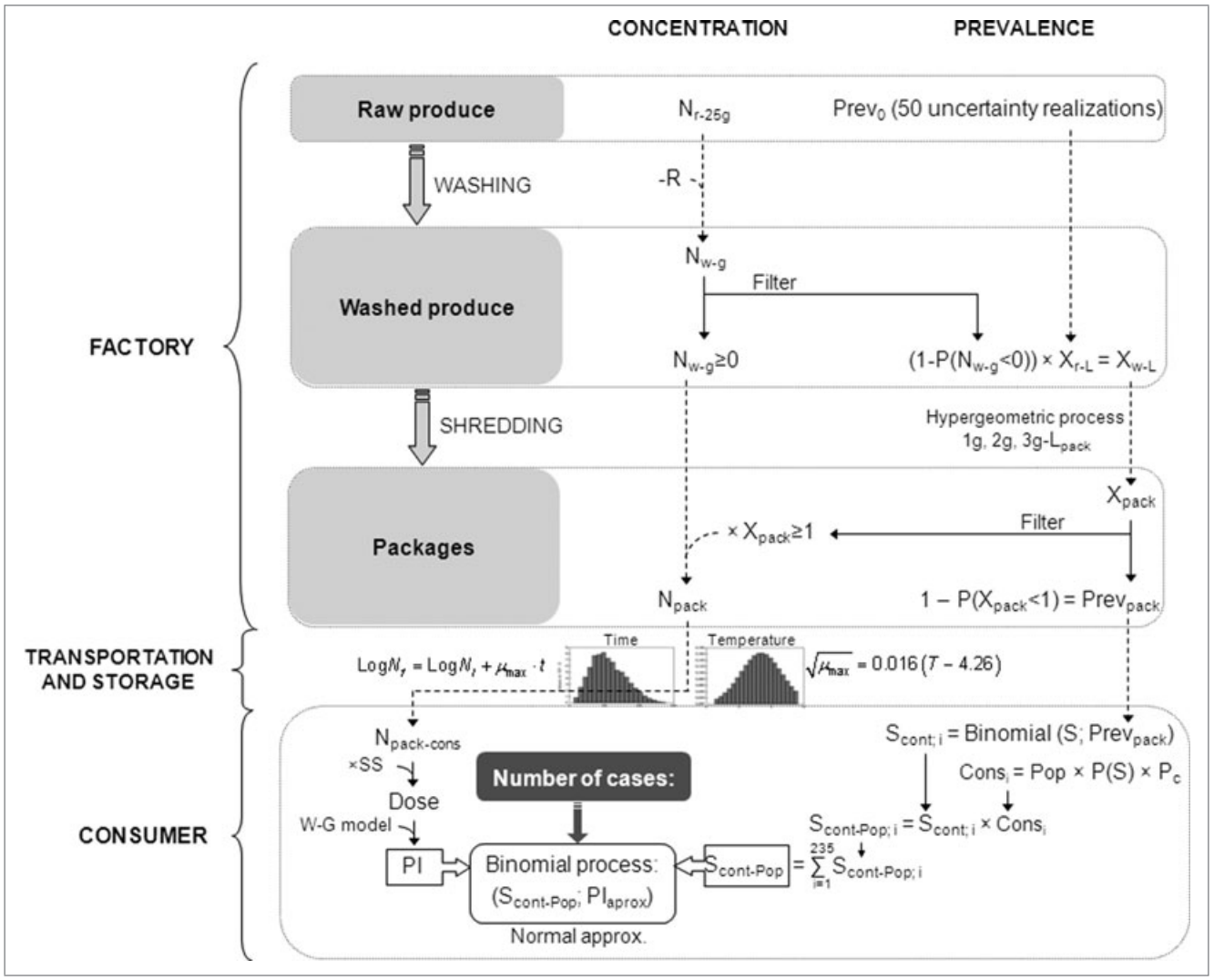

Figure 1–General scheme of the QMRA model of *Listeria monocytogenes* in RTE lettuce salads.

and the most appropriate distribution was selected for subsequent calculations.

The survey carried out (Carrasco and others 2007) also reported the fraction of population consuming fresh-cut leafy green salads (P_c) and that was 75.7%. This information was included in the QMRA, as only this fraction of population is exposed to *L. monocytogenes* via RTE lettuce salads. To quantify the uncertainty associated with P_c, a beta distribution was defined based on the above data (Vose 2008).

Serving size (SS) is a factor strongly related to the number of the pathogen cells ingested through RTE lettuce salads. Table 1 shows the variables, models, and data sources associated with population characteristics to finally estimate the number of contaminated servings consumed by the Spanish population ($S_{cont-Pop}$) (see Table 1).

Status of *L. monocytogenes* in RTE lettuce salads at the time of consumption. The status of *L. monocytogenes* in a food is defined by the prevalence and concentration of the pathogen in the food at the time of consumption. The QMRA model described changes in both parameters from manufacture at the factory to the time of consumption. Figure 2 shows a general food chain of RTE lettuce salads. Steps before manufacture were not considered in the model as it was assumed that there are no controllable factors influencing the status of *L. monocytogenes* in vegetables.

Prevalence ($Prev_0$) and concentration of *L. monocytogenes* in raw vegetables (N_{r-25g}) were the initial inputs of the QMRA model. Prevalence data were taken from the literature (Table 2) and reflected the uncertainty of the QMRA model, together with "the percentage of population that purchases and consumes RTE lettuce salads" (P_c). Regarding concentration of the pathogen, as no information about the level of *L. monocytogenes* in raw vegetables was available in Spain, it was assumed to be equal to the concentration found by Gombas and others (2003) for bagged precut leafy salads (Table 3).

Both prevalence ($Prev_0$) and concentration of *L. monocytogenes* in raw vegetables (N_{r-25g}) were defined separately by 2 cumulative probability distributions (Table 4) by modeling data from Table 2 and 3. Uncertainty of the model ($Prev_0$) was incorporated by sampling 50 values from $Prev_0$ distribution (Latin hypercube sampling), that is, 50 uncertainty realizations of $Prev_0$; each of these values was randomly associated with one of 50 uncertainty realizations of P_c.

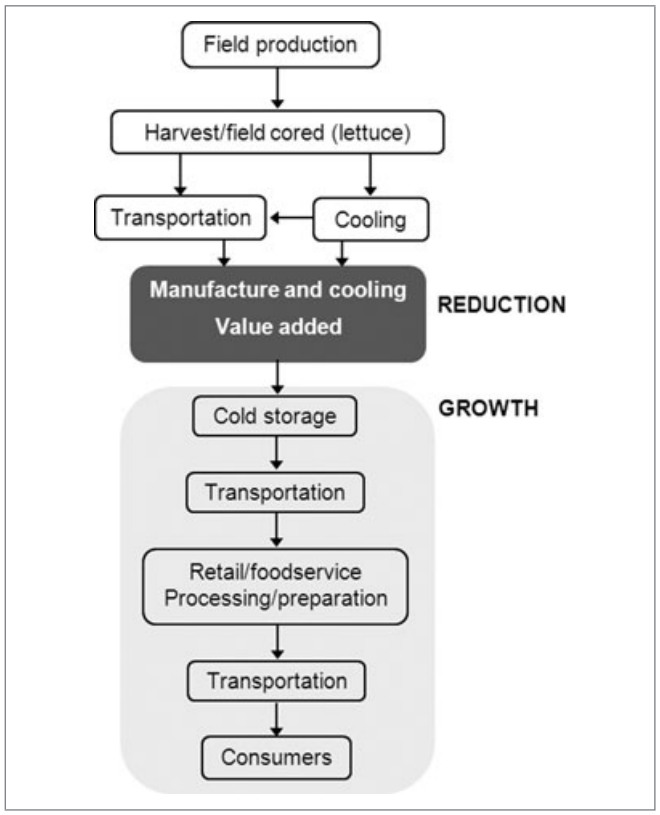

Figure 2–General food chain of RTE lettuce salads.

Table 2–Prevalence of *L. monocytogenes* in vegetables.[a]

Source	Food	Number of samples	Prevalence (%)	F(x)[b]
Lin and others (1996)	Vegetable salads	63	1.6	0.1
Velani and Roberts (1991)	Salad vegetables (intact vegetable)	108	1.8	0.2
Breer and Baumgartner (1992)	Salad vegetables	263	2.3	0.3
Tang and others (1994)	Lettuce (intact vegetable)	28	3.6	0.5
HHS-FDA and USDA-FSIS (2003)	Vegetables	9223	3.6	
Legnani and others (2004)	Raw vegetables	43	6.9	0.6
De Simón and others (1992)	Vegetables	103	7.8	0.7
Harvey and Gilmour (1993)	Raw vegetables	66	10.6	0.8
Arumugaswamy and others (1994)	Leafy vegetables	22	22.7	0.9

[a]The maximum value in the distribution (0.50) was the maximum prevalence we found in the literature (Gunasena and others 1995).
[b]F(x) is the cumulative probability F(x) = $i/(n+1)$, where i is the rank of the observed data point and n is the number of data points (9 surveys) (Vose 2008).

Table 3–Concentration assumed for *L. monocytogenes* in vegetables (Gombas and others 2003).

Concentration (cfu/g)	Concentration (Log_{10} cfu/g)	Concentration (Log_{10} cfu/25 g)	Number of positive samples[a]	f(x)	F(x)
0.04–0.1	(−1.4)–(−1)	0–0.4	17	0.77	0.77
0.1–1	(−1)–0	0.4–1.4	1	0.04	0.82
1–10	0–1	1.4–2.4	1	0.04	0.86
$10–10^2$	1–2	2.4–3.4	2	0.09	0.95
$10^2–10^3$	2–3	3.4–4.4	1	0.04	1

[a]Total number of samples analyzed: 2966.

Raw vegetables were approximated as lettuce heads of 400 g, with location of the potential contamination in the external part of the heads (100 g).

At the factory, it was assumed that a lot size of 250 lettuce heads was manufactured. Two key processes were modeled: washing and shredding (Figure 1). Washing is aimed at reducing the load and/or prevalence of the microbial flora and pathogens. As can be seen in Figure 1, the washing step resulted, on one hand, in a reduction of concentration of the pathogen, $N_{r-25g} \rightarrow N_{w-g}$, that is "concentration in raw produce (log_{10} cfu/25 g)" → "concentration in washed produce (log_{10} cfu/g)," and, on the other hand, in a reduction of the prevalence in the lot in terms of number of contaminated grams, $X_{r-L} \rightarrow X_{w-L}$, that is, "number of contaminated grams of raw product in a lot" → "number of contaminated grams of washed product in a lot." The concentration values of the pathogen in washed produce ($N_{w-g} \geq 0$) were modeled by @Risk software for subsequent calculations; the values $N_{w-g} < 0$ were assumed as absence of the pathogen in the product, due to the disinfectant washing effect. The probability associated with $N_{w-g} \geq 0$, equal to $1 - (N_{w-g} < 0)$, was employed to calculate the actual contaminated grams of lettuce in a lot after washing (X_{w-L}). Washing with chlorinated water was assumed as the sanitary treatment, and data taken from the literature were used to define a normal distribution for reduction (R) of

Table 1–Population characteristics.

Variable	Description (units)	Distribution/model	Source
P_c	Percentage of population that purchases and consumes RTE lettuce salads (%)	Beta(75.7 + 1; 100–75.7 + 1)	Carrasco and others (2007)
SS	Serving size (g)	Cumulative(25; 200; {28;55;123}; {0.5;0.75;0.95})	HHS-FDA and USDA-FSIS (2003)
S	Number of servings consumed per individual and year	Weibull(0.88139; 41.082; RiskShift(3.0003))	Carrasco and others (2007)
$S_{cont;i}$	Number of contaminated servings consumed per individual and year with a consumption profile i (i = 1, 2, 3, . . . , 235 servings per year)	$S_{cont;i}$ = Binomial(S; $Prev_{pack}$)	–
Pop	Spanish population size (either low-risk or high-risk)	Fixed value	INE (2010)
$Cons_i$	Number of consumers of RTE lettuce salads in Spain with a consumption profile i (i = 1, 2, 3, . . . , 235 servings per year)	Consi = Pop × P(S) × P_c	–
$S_{cont\text{-}Pop;i}$	Number of contaminated servings consumed by the Spanish population in a year with a consumption profile i (i = 1, 2, 3, . . . , 235 servings per year)	$S_{cont\text{-}Pop;i} = S_{cont;i} \times Cons_i$	–
$S_{cont\text{-}Pop}$	Number of contaminated servings consumed by the Spanish population in a year	$S_{cont\text{-}Pop} = \sum_{i=1}^{235} S_{cont\text{-}Pop;i}$	–

L. monocytogenes (Table 5). The shredding step was assumed to yield pieces of 1, 2, and 3 g at equal mass proportions, grams 1g-L_L = grams 2g-L_L = grams 3g-L_L. To obtain the number of contaminated pieces of lettuce falling into 200-g packages (1g, 2g, 3g-L_{pack}) a hypergeometric process, approximated with a binomial distribution, was applied. The concentration of *L. monocytogenes* in each pack (log_{10}cfu/pack) (N_{pack}) was obtained for the 50 uncertainty realizations of $Prev_{pack}$. A detailed description of the modeling process of washing, shredding, and distribution in packs is shown in Figure 3.

Table 4–Initial concentration and prevalence of *L. monocytogenes* in raw produce.

Variable	Description (units)	Distribution/model	Source
$Prev_0$[a]	Prevalence of contaminated heads of lettuce	Cumulative(0.01; 0.5; {0.016;0.018;0.023;0.036;0.07; 0.078;0.106;0.227}; {0.1;0.2;0.3;0.5;0.6;0.7;0.8;0.9})	Cumulative distribution from data tabulated in Table 2
$N_{r\text{-}25g}$	Concentration of *L. monocytogenes* in raw produce (log_{10} cfu/25 g)	Cumulative(0; 5.39; {0.4;1.4;2.4;3.4;4.4}; {0.773;0.818; 0.864;0.955;1})	Cumulative distribution from data tabulated in Table 3

[a] Fifty uncertainty realizations of the variable were performed.

Table 5–Manufacture of RTE lettuce salads at the factory.

Variable	Description (units)	Distribution/model	Source
R	Log_{10} reduction of the concentration of *L. monocytogenes* on produce by washing with chlorine (log_{10} cfu/25 g)	Normal (1.96; 0.35; Truncate(1; 3))	Brackett (1987) Zhang and Farber (1996)
$N_{w\text{-}25g}$	Concentration of *L. monocytogenes* in washed produce (log_{10} cfu/25 g)	$N_{w\text{-}25g} = N_{r\text{-}25g} - R$	–
$N_{w\text{-}g}$	Concentration of *L. monocytogenes* in washed produce (log_{10} cfu/g)	$N_{w\text{-}g} = log_{10}(10\text{^}N_{w\text{-}25g}))/25)$ ↓ Fit of distributions to $N_{w\text{-}g} \geq 0$ BetaGeneral(1.045; 2.6407; 0.0010267; 1.8458)	–
$X_{r\text{-}L}$[a]	Number of contaminated grams of raw product in a lot	$X_{r\text{-}L}$[a] = $Prev_0 \times 250^b \times 100^c$	–
$X_{w\text{-}L}$[a]	Number of contaminated grams of washed product in a lot	$X_{w\text{-}L} = (1\text{-}P(Nw\text{-}g < 0)) \times Xr\text{-}L$	–
Grams 1g, 2g, 3g-L_L[a]	Grams of contaminated pieces of lettuce in a lot of 1 g, 2 g and 3 g, respectively, after washing	Grams 1g-$L_L = X_{w\text{-}L}/3$ Grams 2g-$L_L = X_{w\text{-}L}/3 \times 2$ Grams 3g-$L_L = X_{w\text{-}L}/3 \times 3$	–
1g, 2g, 3g-L_{pack}[a]	Number of 1 g, 2 g and 3 g-contaminated pieces of lettuce, respectively, in a package	1g-L_{pack} = Binomial(67; 1g-L_L/(100000/3)[d]) 2g-L_{pack} = Binomial(33; 2g-L_L/(100000/3 × 2)[d]) 3g-L_{pack} = Binomial(22; 3g-L_L/(100000/3 × 3)[d])	–
X_{pack}[a]	Number of contaminated grams of product in a package	X_{pack} = (1g-L_{pack}) + (2g-L_{pack}×2) + (3g-L_{pack}×3)	–

[a] Fifty uncertainty realizations of the variable.
[b] Assumption: 250 heads of lettuce in a lot.
[c] Assumption: only the external part of the head of lettuce is contaminated, namely 100 g.
[d] Assumption: a lot contains 100000 g of lettuce (250 heads of lettuce multiplied by 400 g each).

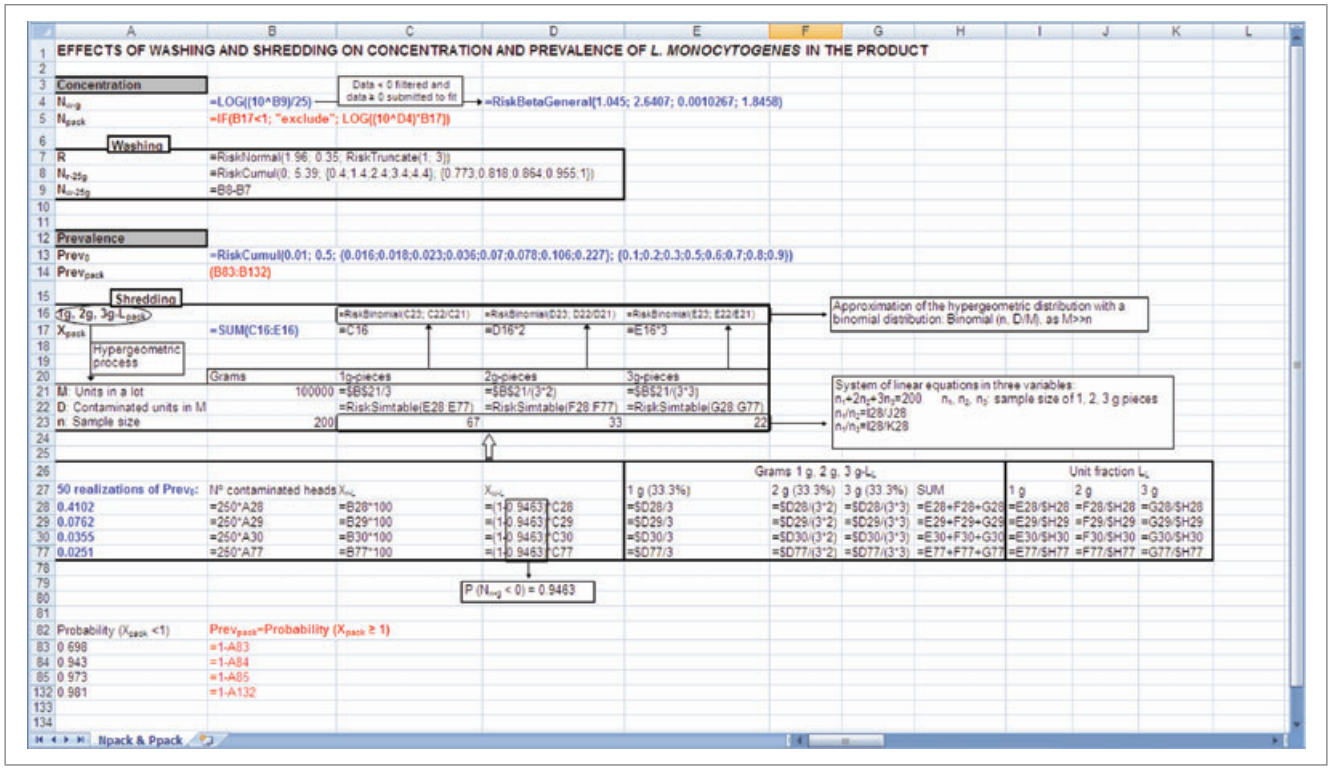

Figure 3–Excel spreadsheet modeling of washing and shredding of lettuce at the factory to calculate $Prev_{pack}$ and N_{pack}.

Table 6–Storage at retail/foodservice—transportation—storage at home.

Variable	Description (units)	Distribution/model	Source
$Temp_R$	Storage temperature at retail/foodserving (°C)	Cumulative(−2; 20; {0;1.6;3.3;5;6.6;8.3;10;11.6;13.3; 15;16.6;18.3}; {0.059;0.109;0.258;0.526;0.734; 0.833;0.932;0.962;0.982;0.990;0.998;0.999})	Audits International (2000)
$time_R$	Storage time at retail/foodserving (h)	Triangular(1; Uniform(2; 9); 37)	Audits International (2000)
$Temp_T$	Mean change in temperature during transportation from store to home (°C)	$Temp_T = (-1.318 * (time_T^2)) + (5.8701 * time_T)$ $R^2 = 0.97$[a]	Audits International (2000)
$time_T$	Transportation time from store to home (h)	Triangular(0; 1; 2.5)	Audits International (2000)
$Temp_H$	Storage temperature at home (°C)	Normal(6.78; 2.56; Truncate(1; 11.3))	Carrasco and others (2007)
$time_H$	Storage time at home (h)	Triang(12; Uniform(72; 96); Uniform(192; 288))	HHS-FDA and USDA-FSIS (2003)

[a]Quadratic equation built from data provided by Audits International (2000).

After packaging of RTE lettuce salads, they are stored and transported at refrigeration temperatures until reaching retail points or foodservice centers. We considered the effect that these stages could have on the concentration of the pathogen in the food negligible, as RTE lettuce salads are rapidly delivered after manufacture. However, the subsequent steps, that is, storage at *retail/foodservice* points, *transport* from these to home, and *storage at home*, were modeled to estimate the growth of *L. monocytogenes*. For this, the Ratkowsky type predictive model of Koseki and Isobe (2005a) was applied on account of its food specificity (model lettuce-based):

$$\sqrt{\mu_{\max}} = 0.016 \cdot (T + 4.26) \quad (1)$$

where $\mu_{\max}$ is the maximum growth rate ($\log_{10}$ cfu/h) and T is temperature (°C). In this equation, temperature distributions for the steps *retail/foodservice* ($Temp_R$) and *storage at home* ($Temp_H$) were introduced; in the case of *transport* ($Temp_T$), an equation describing the increase of temperature (°C) as a function of time was employed. Table 6 shows the probability distributions employed and reference sources.

The $\mu_{\max}$ obtained for each step (*retail/foodservice*, *transport,* and *storage at home*) was introduced in the equation of exponential growth (Eq. 2) to calculate the concentration of cells after each step.

$$\mathrm{Log}N_f = \mathrm{Log}N_i + \mu_{\max} \cdot t \quad (2)$$

where N_f is the concentration of cells (cfu/g) at the end of the stage considered, N_i is the concentration of cells (cfu/g) at the beginning of the stage, $\mu_{\max}$ is the maximum growth rate ($\log_{10}$ cfu/h), and t is the duration of the stage (h).

In order to limit the exponential growth of *L. monocytogenes* at a certain level (stationary phase), an equation for the maximum population density (MPD) given by Koseki and Isobe (2005a) was applied:

$$MPD = 0.037 \cdot T + 12.434, \quad (3)$$

where MPD is the maximum population density (ln cfu/g) and T is temperature (°C).

It is worth to describe briefly the modeling process for $Temp_H$. As data source, temperature profiles reported by Carrasco and others (2007) were used. By using Eq. 1, together with Eq. 2, the increase of the level of *L. monocytogenes* at each temperature profile was calculated. Subsequently, the "effective" static temperature causing the same increase as above was calculated by substituting $\mu_{\max}$ from Eq. 2 in Eq. 1, as can be seen in Eq. 3:

$$T_{eff} = \frac{\sqrt{\frac{\log_{inc}}{t}}}{0.016} - 4.26 \quad (3)$$

where T_{eff} is the "effective" static temperature (°C), t is the time during which the temperature was recorded (h), and $\log_{inc}$ is the increase of *L. monocytogenes* ($\log_{10}$ units) at each temperature profile. A probability distribution for $Temp_H$ was fitted to the overall T_{eff} data (Table 6).

The duration of each step ($time_R$, $time_T$, and $time_H$) was described by means of triangular distributions, detailed in Table 6. A negative correlation between time and temperature of storage at home was assumed, as it is reasonable to think that the higher the temperature, the faster the spoilage of RTE lettuce salads and the shorter the storage time at home. A negative correlation value of 0.25 was used (HHS-FDA and USDA-FSIS 2003).

Hazard characterization

Codex Alimentarius (1999) defined hazard characterization as the "qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with the hazard. For the purpose of microbiological risk assessment, the concerns relate to microorganisms and/or their toxins."

In the present QMRA, the Weibull-Gamma (W-G) model developed by Farber and others (1996) was used. The W-G model is a "single-hit" model which assumes that, whichever the dose is, there is always (at least in a mathematical sense) a nonzero probability of infection or illness, as infections may result from the survival of a single, viable, infectious, pathogenic organism (FAO/WHO 2004a). The W-G model is:

$$\mathrm{PI} = 1 - [1 + (D^b)/\beta]^{-\alpha} \quad (5)$$

where PI is the probability of illness for an individual exposed to D cells, D is the dose (cfu/serving), b is a parameter which determines the shape of the individual dose response curve, and α and β are the parameters of the Gamma distribution describing the heterogeneity host/pathogen. The values considered for the parameters were: $\alpha = 0.25$, $b = 2.14$, and $\beta = 10^{15.26}$ or $10^{10.98}$, depending on whether the characterization was performed in low-risk or high-risk population, respectively.

Risk characterization

While the order of presentation of the previous risk assessment steps does not strictly follow a chronological order, risk characterization is, undoubtedly, the last step. According to Codex Alimentarius (1999), risk characterization is "the process of determining the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization, and exposure assessment."

The risk of listeriosis in the Spanish population was estimated by integrating the results from the previous steps, as shown in

Table 7–Hazard and risk characterization.

Variable	Description (units)	Distribution/model	Source
PI	Probability of illness per contaminated serving	$PI = 1 - [1 + (D^b)/\beta]^{-\alpha}$ (W-G model)	Farber and others (1996)
PI_{aprox}	Normal approximation to PI	$Normal(\mu;^a \sigma^b/\sqrt{S_{cont\text{-}Pop}})$	Vose (2008)
Number of cases	Number of cases of listeriosis in low-risk/high-risk population	Normal approximation of the binomial distribution: $(S_{cont\text{-}Pop}; PI_{aprox}) = Normal(S_{cont\text{-}Pop} \times PI_{aprox}; \sqrt{S_{cont\text{-}Pop} \times PI_{aprox} \times (1 - PI_{aprox})})$	Vose (2008)

[a]Median of PI.
[b]Standard deviation of PI.

Figure 1. The number of cases of listeriosis among low-risk and high-risk populations was simulated (10000 iterations) for the 50 uncertainty realizations of $Prev_{pack}$ obtained in the exposure assessment above. At each uncertainty calculation, a random value of P_c (uncertainty variable) was assigned. The probability of illness for an individual exposed to *D* cells (PI) was assumed to follow a normal distribution and was described according to the central limit theorem (Table 7). The number of listeriosis cases per year followed a binomial process; however, because of the high number of servings consumed ($S_{cont\text{-}Pop}$), a normal approximation of the binomial distribution was used (Table 7).

Sensitivity Analysis Procedure

Sensitivity analysis is a tool that allows determining the effects that inputs have on model outputs, such us quantifying how sensible an output is to changes of inputs. A sensitivity analysis was performed for the outputs *number of cases in low-risk population* and *number of cases in high-risk population*. The inputs selected for the analysis were those probability distributions directly introduced in the mathematical model: $N_{pack\text{–}cons}$, $Temp_R$, $Temp_H$, $time_R$, $time_T$, $time_H$, and SS. The sensitivity analysis method applied was that provided by @Risk Professional© software for Advanced Sensitivity Analysis, in which a number of simulations are run for each input. With this tool, several inputs "steps" are fixed by the user, and a simulation is performed at each "step." In the present work, the inputs "steps" were set at various percentiles (1st, 5th, 25th, 50th, 75th, 95th, and 99th), and each one was simulated with 500 iterations (Latin hypercube sampling).

Risk Management Options and Implementation Procedure

As a desirable, general goal, a level of 100 cfu/g in the product at the time of consumption was set in the QMRA model (baseline model), in compliance with Regulation (CE) N° 2073/2005, which establishes a microbiological criterion of 100 cfu/g throughout the shelf-life of *ready-to-eat foods able to support the growth of L. monocytogenes, other than those intended for infants and for special medical purposes*. The achievement of this goal was evaluated in terms of number of cases of listeriosis per year in Spain.

Examples of RM options that would benefit from the availability of a risk assessment to achieve specific RM goals were reported by FAO/WHO (2006) (Table 8). We went through these goals and options and analyzed their application in reducing the risk of listeriosis by RTE lettuce salads consumption. We tested 4 hypothetical RM measures and, also, the compliance with Regulation 2073/2005 (100 ufc/g throughout the shelf-life of products) in order to reduce the disease burden.

Risk communication program strategy

We assumed that an effective risk communication program would result in a 50% reduction of the probability of consumption of RTE lettuce salads by the high-risk population (P_c for high-risk population was halved).

Table 8–Examples of risk management (RM) goals and options proposed by FAO/WHO (2006) and measures adopted in this work.

RM goals	RM options available to achieve these goals	Measures adopted in this work
Avoid exposure to a specific food	Ban production and/or harvest Ban importation	
Reducing consumer exposure to hazards in specific foods	Informing vulnerable consumers (and care-givers) not to eat specific foods Preventing a food from entering the food chain	Prevent susceptible consumers from exposure to RTE lettuce salads (by means of Risk Communication)
Control initial levels of hazards in raw ingredients derived from primary production or those ingredients entering the processing environment	Using microbiological criteria to identify and reject unacceptable ingredients Selecting ingredients that have undergone reduction treatment Development and implementation or review of current Codes of Practice addressing GAP/GMP/GHP/HACCP	Application of sampling plans for microbiological criteria (absence in 25 g) at primary production (raw vegetables)
Prevent an increase in contamination and the level of a hazard in a food	Reduce additional (re)contamination and growth of pathogens	Use of specific mixture of gases in packages of RTE salad at the factory level
Reduce level of hazard in a food	Implementation of selected processing operations which eliminates or reduces pathogens	
Remove pathogen from a food	Implementation of processing operations which remove pathogens	
Do nothing (maintain status quo)	Not applicable	

Implementation of microbiological criteria at primary production

Three microbiological criteria were tested ($n = 10$ $c = 0$, $n = 20$ $c = 0$, and $n = 30$ $c = 0$; absence in 25 g in all 3 of them) at primary production, which was implemented by selecting only those prevalence data sources from Table 2 whose confidence distribution showed more certainty in obtaining a certain value than the microbiological criteria tested. The confidence (or uncertainty) of both prevalence sources and microbiological criteria was described by Beta distributions presented in Table 9, and named $UPrev_{source}$, $UPrev_{n10}$, $UPrev_{n20}$, and $UPrev_{n30}$, the latter 3 referring to the microbiological criteria tested. Implementation of microbiological criteria at primary production was modeled as follows. From the Beta distributions of microbiological criteria,

Table 9–Management option: Sampling plan at primary production.

Variable	Description (units)	Distribution/Model	Source
$UPrev_{n10}$	Uncertainty of the prevalence of a lot which fulfills a microbiological criterion n = 10 c = 0, absence in 25 g	$UPrev_{n10}$ = Beta(0 + 1; 10–0 + 1)	–
$UPrev_{n20}$	Uncertainty of the prevalence of a lot which fulfills a microbiological criterion n = 20 c = 0, absence in 25 g	$UPrev_{n20}$ = Beta(0 + 1; 20–0 + 1)	–
$UPrev_{n30}$	Uncertainty of the prevalence of a lot which fulfills a microbiological criterion n = 30 c = 0, absence in 25 g	$UPrev_{n30}$ = Beta(0 + 1; 30–0 + 1)	–
$UPrev_{source}$	Uncertainty of the prevalence found by the corresponding source:	Beta(1 + 1; 63–1 + 1) Beta(2 + 1; 108–2 + 1) Beta(6 + 1; 263–6 + 1) Beta(1 + 1; 28–1 + 1) Beta(332 + 1; 9223–332 + 1) Beta(3 + 1; 43–3 + 1) Beta(8 + 1; 103–8 + 1) Beta(7 + 1; 66–7 + 1) Beta(5 + 1; 22–5 + 1)	[a]Lin and others (1996) [b]Velani and Roberts (1991) [c]Breer and Baumgartner (1992) [d]Tang and others (1994) [e]HHS-FDA and USDA-FSIS (2003) [f]Legnani and others (2004) [g]De Simón and others (1992) [h]Harvey and Gilmour (1993) Arumugaswamy and others (1994)
$Prev\text{-}M_{n10}$	Prevalence defined by various sources whose 95% confidences are equal or greater than the 95% confidence of $UPrev_{n10}$ for the same value	Cumulative (0.00000103; 0.576; {0.016;0.018;0.023;0.036; 0.069;0.078;0.106}; {0.11;0.22; 0.33;0.55;0.66;0.77;0.88})[i]	Data from[a,b,c,d,e,f,g,h] sources in this table
$Prev\text{-}M_{n20}$	Prevalence defined by various sources whose 95% confidences are equal or greater than the 95% confidence of $UPrev_{n20}$ for the same value	Cumulative (0.00000125; 0.366; {0.016;0.018;0.023;0.036}; {0.2;0.4;0.6;0.8})[i]	Data from [a,b,c,e] sources in this table
$Prev\text{-}M_{n30}$	Prevalence defined by various sources whose 95% confidences are equal or greater than the 95% confidence of $UPrev_{n30}$ for the same value	Cumulative (0.00000213; 0.301; {0.016;0.018;0.023;0.036}; {0.2;0.4;0.6;0.8})[i]	Data from [a,b,c,e] sources in this table

[a,b,c,d,e,f,g,h]Source references used to build the probability distribution of $Prev\text{-}M_{n10}$.
[a,b,c,e]Source references used to build the probability distributions of $Prev\text{-}M_{n20}$ and $Prev\text{-}M_{n30}$.
[i]Minimum and maximum values of the cumulative distributions $Prev\text{-}M_{n10}$, $Prev\text{-}M_{n20}$, and $Prev\text{-}M_{n30}$ are the minimum and maximum values of the simulated distributions $UPrev_{n10}$, $UPrev_{n20}$, and $UPrev_{n30}$, respectively (10000 iterations).

a value was selected deemed to be confident enough, the 95th percentile value. This value was introduced as *target value* in the data sources distributions, and the corresponding percentile was evaluated: if greater than 95%, such data sources were selected for defining the initial distribution of prevalence; if lower, they were not selected. With this procedure, only those prevalence data sources that, in theory, satisfied the microbiological criterion tested at 95% confidence were selected. From this selection, different initial cumulative distributions for prevalence (equivalent to $Prev_0$ in the baseline model) were built: $Prev\text{-}M_{n10}$, $Prev\text{-}M_{n20}$, and $Prev\text{-}M_{n30}$ (Table 9).

Reduction of shelf-life

A reduction of shelf-life was assumed by truncating the distribution of $time_H$ at 4, 5, 7, 8, 9, 10, 11, and 12 d.

Change in the packaging atmosphere of RTE lettuce salads

With this measure, manufacture of packages of RTE lettuce salads with injection of gases was assumed. We used our previous results (Carrasco and others 2008) for the use of growth data of *L. monocytogenes* on iceberg lettuce packaged with a typical gas composition (carbon dioxide about 5.5%, oxygen about 3%, and nitrogen about 92.5%), in contrast to the packages of iceberg lettuce (without any injection of gases) upon which the growth model of the baseline model was constructed (Koseki and Isobe 2005b). We investigated what would happen in a worst case scenario of temperature abuse (13 °C) during the stages of storage at retail, transportation, and storage at home. To allow for comparison, the same temperature (13 °C) was applied in our baseline model through the same stages. From our previous work (Carrasco and others 2008), a μ_{max} = 0.019 $\log_{10}$ cfu/h and a MPD = 7.40 $\log_{10}$ cfu/g were used.

As a result of implementation of the 4 RM measures, the QMRA model was modified in 4 different ways, and each modified QMRA model was simulated with 10000 iterations. The resulting number of cases of listeriosis per year was estimated for low-risk and high-risk population.

Results and Discussion

Quantitative microbiological risk assessment model

Population characteristics. The low-risk and high-risk populations in Spain submitted to QMRA were distributed by gender and age, and subpopulations for low-risk and high-risk were calculated on the basis of the fractions reported by FAO/WHO (2004b). From the population size submitted to risk analysis, namely, 45282830, a fraction of 0.23 corresponded to high-risk population.

The number of servings consumed per individual and year (S) was described by a Weibull distribution (RMSE = 2.82×10^{-6}) (Figure 4), showing that consumption of RTE lettuce salads in Spain is generally low.

In contrast with Spanish consumption, in Belgium, the consumption frequencies reported (Ragaert and others 2004) were distributed as follows: 57.1% purchased minimally processed vegetables or packaged fruits *once a week*, 35.4% *once a month*, and only 7.5% *less than once a month*. These frequencies are opposite to those used in this study to model *S* (Carrasco and others 2007): 7.4% *more than twice a week*, 17.3% *once or twice a week*, 29.6% *once or twice a month,* and 45.7% *occasionally*. In this work, taking into account the Weibull distribution for *S*, the fraction of the population consuming lettuce salads (P_c) and the population size submitted to risk assessment, the estimated number of servings consumed per year in Spain was 1.37×10^9, which would mean 1.71 kg per capita in a year (assuming an SS of 50 g, which was the mean in this work). For this calculation, it was assumed that both low-risk and high-risk populations follow the same consumption pattern S_i. This number is in accordance with the consumption reported by Lobo and González (2006) in Spain (annual consumption = 1.5 to 2 kg per capita of minimally processed produce in 2005), and in contrast with consumption patterns of other countries, such as

France, with 6 kg per capita, or the United States, with 30 kg per capita (Lobo and González 2006).

Prevalence and concentration of *L. monocytogenes* in the product. Regarding the status of *L. monocytogenes* in the product, 2 main facts are remarkable along the food chain: (1) reduction of the concentration and prevalence at the factory, and (2) increase of the concentration of the pathogen during transport and storage (Figure 2). Reduction was achieved by washing the raw material at the factory. The simulated concentration of the pathogen after washing ($N_{w-g} \geq 0$) was defined by a BetaGeneral distribution (χ^2 test; $P = 0.1903$), as shown in Figure 5, which was introduced in the QMRA model for the subsequent step, that is, calculation of the concentration of the pathogen in 200-g packages (N_{pack}).

The decrease of prevalence during washing was a result of assuming that simulated values of $N_{w-g} < 0$ were free of *L. monocytogenes*. These values comprised a cumulative probability = 0.9463, whose complement was multiplied by the contaminated grams in the lot being processed (X_{r-L}) to yield X_{w-L}, as shown in Figure 1 and 3. Shredding and distribution of lettuce in 200-g packages resulted in $Prev_{pack}$ (50 uncertainty realizations) which ranged from 0.0074 to 0.3428, with a median = 0.0269. Figure 6 represents the transition from $Prev_0$ to $Prev_{pack}$, as a result of the processes taking place at the factory.

During storage at *retail*, *transport,* and *storage at home*, the time and temperature operating in these stages permitted the growth of *L. monocytogenes* until reaching the concentration in packages at the time of consumption ($N_{pack-cons}$). Throughout the model, the variables dealing with concentration and prevalence of *L. monocytogenes* represented the variability and uncertainty, respectively, of the QMRA model. Codex Alimentarius (1999) recommended the inclusion of uncertainty and variability in risk assessments, although no methodologies were indicated. Vose (2008) pointed out the usefulness of keeping these 2 components separate. Prevalence and concentration of *L. monocytogenes* in foods are often considered to be related properties, particularly at very low concentrations (FAO/WHO 2004b). However, it has been recognized

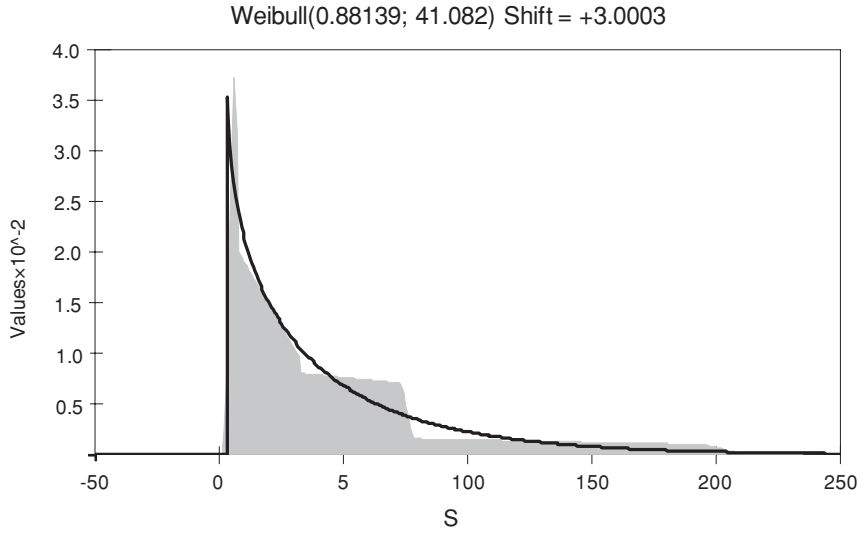

Figure 4–Probability distribution of the number of servings of RTE lettuce salads consumed per individual and year (S).

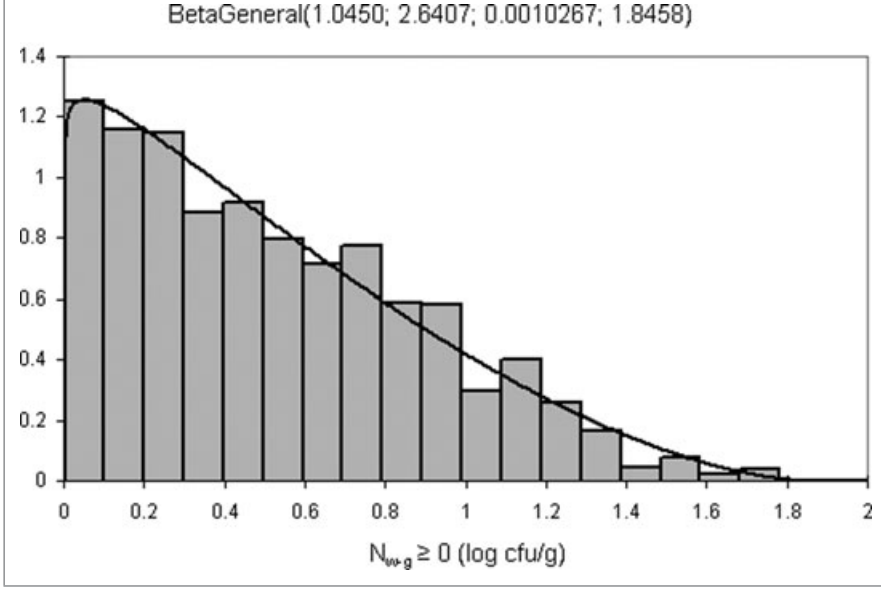

Figure 5–Probability distribution of concentration of *L. monocytogenes* (log_{10} cfu/g) in the washed product ≥ 0 ($N_{w-g} \geq 0$).

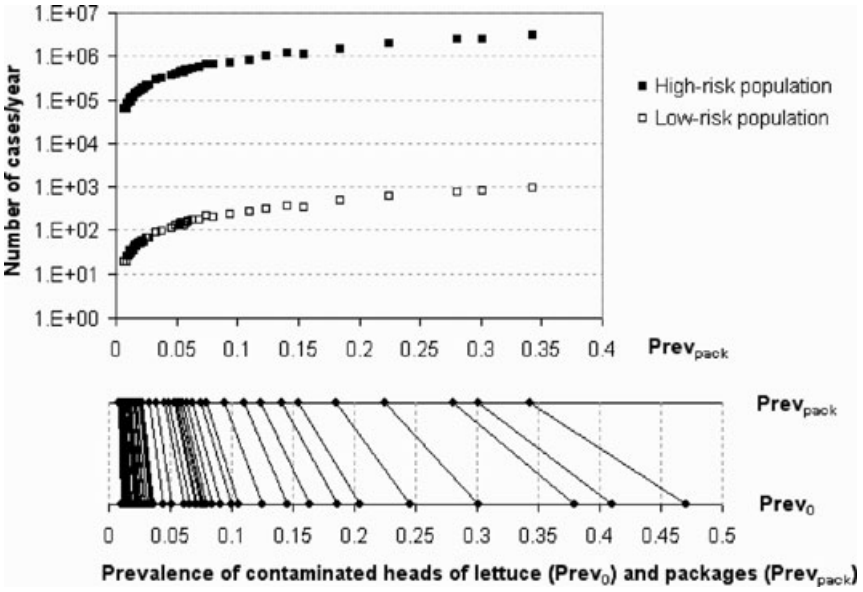

Figure 6–Mean number of cases/year in low- and high-risk populations compared with prevalence of *L. monocytogenes* in packages of lettuce salad ($Prev_{pack}$) and transition from initial prevalence ($Prev_0$) to $Prev_{pack}$.

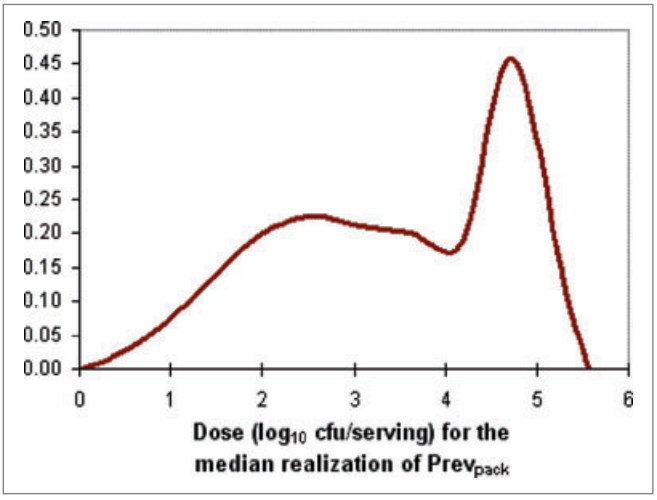

Figure 7–Probability of dose (log_{10} cfu/serving) for the median uncertainty realization of $Prev_{pack}$.

that there is the inconvenience of assuming one distribution to represent both prevalence and concentration, which has been explained with an example (Pérez-Rodríguez and others 2007). In this work, both components were described separately by empirical distributions, since the use of a theoretic or parametric model would be hard to justify with our limited knowledge about the prevalence and levels of *L. monocytogenes*. These types of data are scarce in Spain. Coordination and cooperation between industries and sanitary or public health authorities should be encouraged to provide microbiological data to risk managers and assessors.

At the time of consumption, the amount of *Listeria* cells ingested, the dose, depends on $N_{pack-cons}$ and SS (Figure 1). The simulated dose distribution of *L. monocytogenes* (log_{10} cfu/serving) for the $Prev_{pack}$ median value can be observed in Figure 7.

Figure 7 reveals a concentration of high doses (right zone of the distribution), which is the consequence of extensive growth of *L. monocytogenes* during refrigerated storage, reaching the MPD or close values. In this way, doses >4.5 log_{10} cfu/serving (32% of dose values) corresponded to levels of the pathogen ($N_{pack-cons}$) concentrated between 5.4 and 5.7 log_{10} cfu/g. These results are consistent with those reported from the study by Pérez-Rodríguez and others (2007), concluding that *L. monocytogenes* could grow up to levels of MPD from low initial concentrations because of the recognized psychrotrophic nature of the pathogen.

Hazard and risk characterization. Various attempts have been made to model the probability of infection/illness of *L. monocytogenes*. In this sense, Rocourt and others (2003) revised a number of dose-response relationships reported for *L. monocytogenes* that have been described and are based on different end-points and types of data. Notermans and Hoornstra (2000) pointed out that the dose-response relationships established to date have shown large variations, and their value resides in the estimation of the relative effect of several control options. In this work, the W-G dose–response model was employed because of its goodness of fit to different data sets of various foodborne diseases (Holcomb and others 1999) and its wide application in risk assessments of *L. monocytogenes*. The different PI values given by the W-G model for the 50 uncertainty realizations of the model showed slight variation. For high-risk population, PI means and standard deviations varied between $2.40 \times 10^{-2} - 2.60 \times 10^{-2}$ and $6.10 \times 10^{-2} - 6.30 \times 10^{-2}$, respectively; and for low-risk population, the ranges were 2.45×10^{-6}–2.63×10^{-6} and 9.01×10^{-6}–9.28×10^{-6}, respectively.

Figure 6 shows the mean number of cases of listeriosis/year in low-risk and high-risk populations, corresponding to the 50 uncertainty realizations of $Prev_{pack}$. An uncertainty ranging about 1.7 log_{10} units (multiplicative factor about 39) for the mean number of cases can be observed in subpopulations, owing to the uncertainty of $Prev_{pack}$ estimated. In the high-risk population, the number of cases was 3.5 log_{10} units greater than in the low-risk population. In contrast to our results, Lindqvist and Westöö (2000) obtained the same order of magnitude for both subpopulations. This could be explained by the dose distribution they obtained, with

high-dose levels of up to 8 $\log_{10}$ cfu/serving, and the location of these levels at the right zone of the x-axis of the W-G model (Farber and others 1996). Bemrah and others (1998) obtained about 1 $\log_{10}$ unit difference in the number of cases of listeriosis between high-risk and low-risk subpopulations.

The absolute numbers for the cases of listeriosis (about 10^5 and 10^2 for high-risk and low-risk populations, respectively) (Figure 6) are highly deviating from those observed in Spain in the last years (81, 78, 68, and 100 cases in 2007, 2006, 2005, and 2004, respectively). This could be explained by the growth model used (Koseki and Isobe 2005a) in the QMRA model, which lacked the inclusion of modified atmosphere as a factor influencing the growth of *L. monocytogenes*. The use of modified atmosphere packaging (usually 5 to 10% CO_2, 0.5 to 3% O_2, and the balance of N_2 for RTE lettuce salads) delays the growth of *L. monocytogenes* (Carrasco and others 2008). The extended growth estimated by the model of Koseki and Isobe (2005a) led to high doses and, subsequently, to high individual PI and a large number of cases of listeriosis per year.

Nowadays, it is a common practice to use modified atmosphere packaging for RTE lettuce salads, meaning that the conditions in which the Koseki model was developed do not represent a real situation for RTE lettuce salads. Unfortunately, to our awareness, there is no available growth model for *L. monocytogenes* in lettuce salads including carbon dioxide as a factor, apart from temperature. More research dealing with pathogen growth models in RTE lettuce salads is needed. As growth models will be improved, the QMRA model presented in this paper could be refined. In this work, we demonstrated modeling procedures for risk estimation of listeriosis, leaving aside the absolute risk estimates until more realistic models are available.

Sensitivity analysis

Figure 8 shows tornado graphs for low-risk and high-risk populations corresponding to the median realization of $Prev_{pack}$.

It can be seen that the sensitivity ranking of inputs is the same for both subpopulations. However, in the high-risk population, temperature and time at retail and home ($Temp_R$, $time_R$, $Temp_H$, $time_H$), and the concentration of *L. monocytogenes* in packages at the time of consumption ($N_{pack-cons}$), have more influence on the output than in the low-risk population.

It is observed that SS is a factor strongly related to the number of cases; however, it cannot be conceived of as an RM option to reduce the disease burden. Quite to the contrary, the consumption of vegetable, RTE or fresh, is being promoted by health initiative campaigns such as the "Fruits & Veggies—More Matters^{TM}" program (Fruits & Veggies—More Matters 2010).

$Temp_H$ was the 2nd input that mostly influenced the variation in the mean number of cases. Temperature is a primary factor in controlling the rate of growth of *L. monocytogenes* (Buchanan and others 1989). It seems to be adequate to develop programs for consumer education as a Risk management strategy. The risk assessment carried out by the HHS-FDA and USDA-FSIS (2003) identified *refrigerated storage temperature* as one of the 5 broad factors that affect consumer exposure to *L. monocytogenes*. The others were: *amount and frequency of consumption of RTE food*, *frequency and levels of L. monocytogenes in RTE food*, *potential of the food to support the growth of the pathogen during refrigerated storage,* and *duration of refrigerated storage*.

The 3rd and 4th QMRA inputs in tornado graphs were $time_H$ and $N_{pack-cons}$, respectively. They were considered as potential RM options, as they may be more feasible to be reduced, from a public perspective, than $Temp_H$. The level of contamination in the food product reported by Lindqvist and Westöö (2000) was the factor to which the PI was most sensitive. However, these authors did not model the processes taking place along the food chain, that is, temperature, time, or other factor that may affect the status of the pathogen in the product.

Risk management measures to reduce disease burden

Together with other tools, such as epidemiology-based tools and economic analysis, risk assessment can provide a sound scientific foundation for "risk-based" management systems (FAO/WHO 2006).

The QMRA model developed (baseline model) was modified in order to incorporate all RM options stated previously.

A hypothetical and ideal situation of 100% compliance with Regulation (CE) N° 2073/2005 would mean that the concentration of *L. monocytogenes* in the product at the time of consumption is 100 cfu/g, in other words, a value for $N_{pack-cons}$ of 4.30 $\log_{10}$ cfu/package (a package = 200 g).

By selecting only those simulated values of $N_{pack-cons} \leq 4.30$, the PI mean and standard deviation would decrease until 2.72×10^{-5} and 1.28×10^{-4}, respectively, for the low-risk population, and 1.43×10^{-9} and 6.76×10^{-9}, respectively, for the high-risk population. These numbers are around 2 to 3 $\log_{10}$ units lower than those reported above in the baseline model. Lindqvist and Westöö (2000), in demonstrating this, found 2 orders of magnitude difference by using the exponential dose–response model for PI. In our work, the new PI values calculated were implemented in the QMRA model for the case of $Prev_{pack}$ median, resulting in a mean number of cases of 4×10^{-2} and 244 cases for low-risk and high-risk populations, respectively. If, instead of $Prev_{pack}$, taking the prevalence value reported by the Spanish Zoonoses Report of 2008 (EFSA 2010) (2.1 positive vegetable samples out of 47), the estimated number of cases would be practically the same (5.75×10^{-2} and 350 cases for low- and high-risk populations, respectively). These numbers approximate the burden of the disease in Spain. Nonetheless, it should be borne in mind that listeriosis cases may be attributed to other sources (not only RTE lettuce salads), and also, the number of cases are usually underreported.

In our baseline model, the level 100 cfu/g is exceeded during the shelf-life of RTE lettuce salads, given the growth model employed, and the temperature and time data sets assumed in the 3 growth stages (retail, transport, and storage at home). The level of 100 cfu/g corresponded to the 51.6% percentile of the simulated data of $N_{pack-cons}$ in the baseline model.

In the light of these results, it seems that the hypothetical and ideal situation above is actually achieved in Spain. Luckily, it might be the case, and our baseline model may overestimate the risk of listeriosis due to the assumptions made (for example, the use of the growth model without modified atmosphere packaging). However, if desired to model the food chain of foodstuff at the maximum extent (from primary production to consumption), a decision has to be made in relation to data gaps, acquiring the RM measures is more important than the absolute values of risk from the baseline model.

The public health benefits from implementation of the RM measures adopted in this work are presented in Table 10. Reductions in the number of cases in low-risk and high-risk populations were practically equal, except for the 1st and 3rd RM measure. In the latter case, *"Prevent high-risk consumers from consumption of RTE*

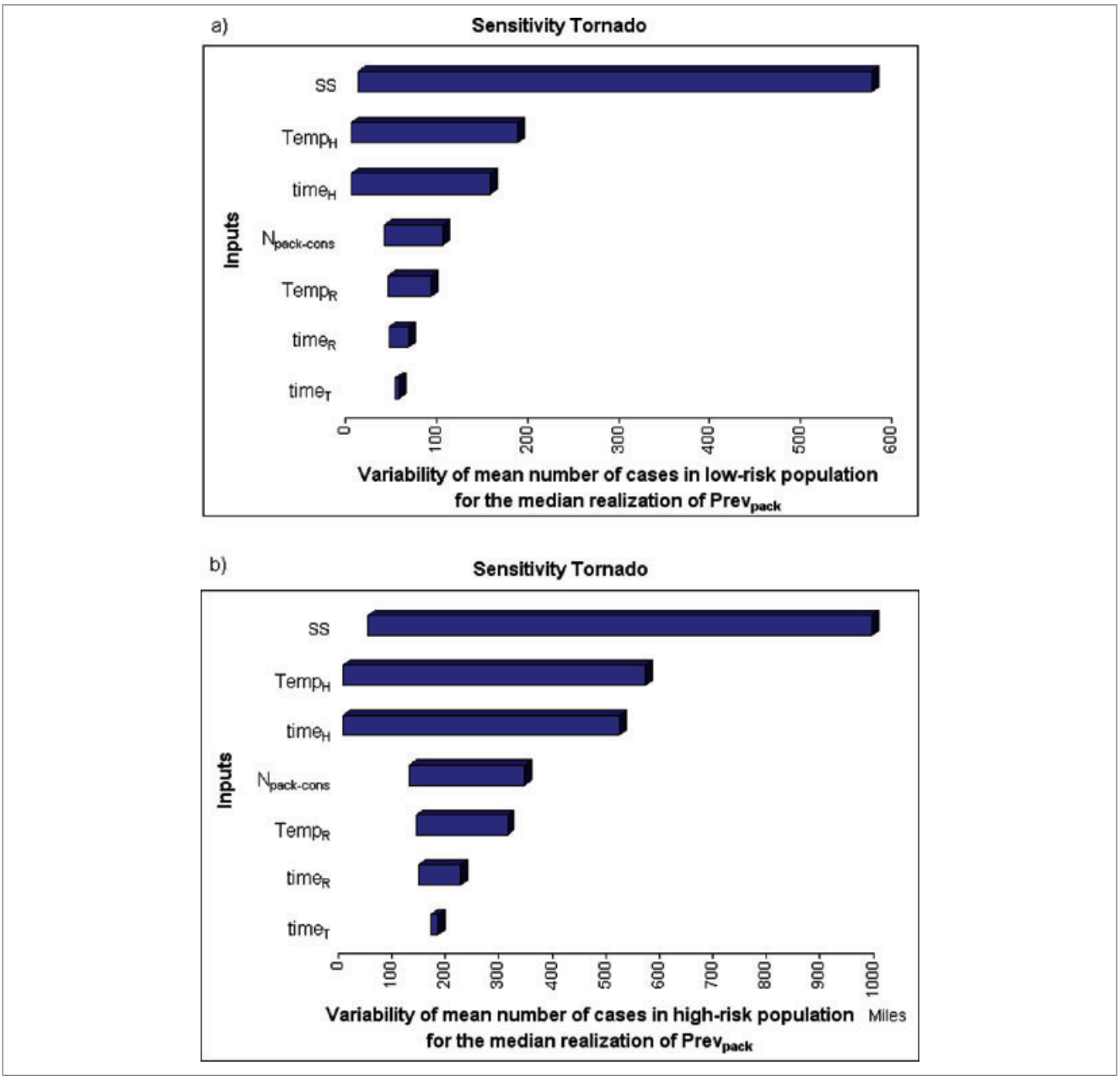

Figure 8–Tornado graphs of sensitivity of the mean number of cases/year to various inputs in (a) low-risk population and (b) high-risk population.

lettuce salads," the reason is obvious; in the former, it is explained below.

The RM measure which produced the major reduction in the number of cases was the first measure adopted, namely, the use of a specific mixture of gases, where $N_{pack-cons}$ values decreased. Among the different gases that could be employed, carbon dioxide has been widely studied as an inhibitor of the growth of *L. monocytogenes* (Bennik and others 1996; Francis and O'Beirne 1998).

The challenge test carried previously by our research group (Carrasco and others 2008) showed that the effect of the mixture of gases applied retarded the growth (lower maximum growth rate) and extended the MPD. The maximum growth rate applied in this RM measure (0.019 $\log_{10}$ cfu/h at 13 °C) resulted in a distribution of $N_{pack-cons}$ with generally lower values than those of the baseline model; for example, the percentage of values below 4.30 $\log_{10}$ cfu/package (100 cfu/g) were 85.9% and 51.6% for the modified model and the baseline model, respectively. Notwithstanding, given the new MPD applied in the modified model, certain percentages of $N_{pack-cons}$ values (1.5%) were greater than the MPD in the baseline model at 13 °C (5.6 $\log_{10}$ cfu/g). This percentage was responsible for <1% of doses, higher in the modified model (between 5.6 and 6.9 $\log_{10}$ cfu/serving) than in the baseline model. These levels of high doses are located in the right zone of the W-G model (Farber and others 1996) whose curve is different for low-risk and high-risk populations; for the low-risk, the dose–response curve is, at these dose levels, in the exponential form, while in the case of the high-risk population it has already reached a "plateau" near the maximum PI. In this way, despite an important reduction is achieved in both subpopulations by the *"Use of specific mixture of gases,"* the proportion of high doses in

Table 10–Ranking of risk management measures according to the reduction of burden of listeriosis in the population of Spain.

RM measures adopted in this work	Reduction percentage (%)		
	Low-risk population	High-risk population	Total population
1. Use of specific mixture of gases	66	95	95
2. Reduction of shelf-life: 4-d $time_H$	85	84	84
3. Prevent high-risk consumers from consumption of RTE lettuce salads	0	75	75
4. Reduction of shelf-life: 5-d $time_H$	64	62	62
5. Microbiological criterion at primary production: $n = 30$; $c = 0$; absence in 25 g	44	44	44
6. Microbiological criterion at primary production: $n = 20$; $c = 0$; $c = 0$; absence in 25 g	42	43	43
7. Reduction of shelf-life: 6-d $time_H$	42	40	40
8. Reduction of shelf-life: 7-d $time_H$	26	24	24
9. Reduction of shelf-life: 8-d $time_H$	13	11	11
10. Microbiological criterion at primary production: $n = 10$; $c = 0$; $c = 0$; absence in 25 g	6	8	8
11. Reduction of shelf-life: 9-d $time_H$	5	4	4

the modified model resulted in a relatively important risk increase in the low-risk population, yielding a lower net reduction of the number of cases than in the high-risk population.

The reduction of $time_H$ by shortening the shelf-life has shown to be very important in reducing the number of cases (Table 10).

The application of microbiological criteria at primary production allowed the decrease of initial prevalence, as those "lots" (data sources from Table 2) resulting in lower confidence level than 95% for the value representing the 95% percentile in the microbiological criteria were rejected. The procedure followed in this work to test how microbiological criteria in primary production could affect a risk assessment output is of high importance for sanitary public health authorities, and, to our concern, no attempt has been made to show how to evaluate microbiological criteria in terms of public health.

Garrido and others (2010) showed that, in general terms, the most relevant scenario to reduce the burden of listeriosis in different RTE products (sliced-cooked meat and smoked fish) was the combination *short time–low temperature* storage. However, they did not test other RM measures such as the use of modified atmosphere packaging, prevention of consumption, or microbiological criteria application.

A number of limitations can be identified in the present QMRA model, which has been summarized below:

- Exposure assessment step: prevalence and concentration of *L. monocytogenes* in raw vegetables were taken from foreign sources, with just one exception, namely, De Simón and others (1992); washing was assumed to yield batches of product with lower prevalence than that of the intact product; a model for shredding and distribution of lettuce in packages was assumed; cross-contamination was not considered; the growth model applied did not consider modified atmosphere packaging; frequency of consumption of the Spanish population was modeled based on a limited interview, which was generalized for the entire Spanish population; SS data were assumed from American sources; low-risk and high-risk populations have the same consumption pattern.
- Hazard characterization: a "single-hit" model was employed (W-G). The immune response of individuals to the hazard was not taken into account; it was assumed that all strains isolated from food have the same potential to cause listeriosis.

To date, all risk assessment performed has unavoidable limitations due to the scarcity of data and uncertainty about parameter values. Thus, it appears that the effects derived from application of RM measures are more valuable than the absolute value of QMRA outputs.

An important issue when evaluating different RM options is the cost of implementation. Todd and Roberts (1996) listed issues which need consideration for estimating the costs of foodborne illnesses. It is a matter of the competent authorities to balance the cost of both illness and implementation of RM measures, and then make a decision.

Conclusion

With this work, novel modeling approaches have been made in several steps of RTE lettuce salads production, such us manufacture or introduction of microbiological criteria in primary production. Only when modeling a food chain to maximum extent can it be known how parameters like prevalence or concentration of pathogens can change along the different steps of the food chain.

Although disease burden of listeriosis estimated by the QMRA model does not match what is actually observed in Spain due to numerous assumptions and approximations, the model structure is highly valuable, and further research and collection of data would simply fill in the gaps adequately.

Results in reduction of listeriosis cases by application of RM measures deserve special attention. A ranking of measures was presented, showing that the measure "the use of a mixture of gases" appears to be the most important. Measures such us application of microbiological criteria are ranked in the 5th, 6th, and 10th position. Traditionally, microbiological criteria have been established to improve food safety. Nevertheless, the work shows that other measures should be explored first. At the same time, costs and social implications of RM measures application should be evaluated.

More research and cooperation between different stakeholder organizations are needed in order to progressively improve QMRA models. With this work, a breakthrough has been made with regard to risk assessment and management procedures and implementation.

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