Evaluation of the sensitivity of microbiological criteria for *Listeria monocytogenes* in detecting unsafe food according to the prevalence of the pathogen and the shelf-life of the food

Jean-Christophe Augustin*

Service d’Hygiène et Industrie des Denrées Alimentaires d’Origine Animale, Ecole Nationale Vétérinaire d’Alfort, 7 avenue du Général de Gaulle, F-94704 Maisons-Alfort Cedex, France.

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Abstract

The sensitivity of criteria in detecting unsafe food was studied for a hypothetical ready-to-eat food supporting growth of *Listeria monocytogenes* during storage. The effects of the prevalence of the pathogen and of the duration of the shelf-life on the probability for servings to be unsafe and on the probabilities of detecting unsafe food were evaluated. The results obtained in this example show that the probability for servings to be unsafe increases with the prevalence and the shelf-life while the probabilities of detecting unsafe food depend above all on the prevalence of the pathogen. The current criteria used for *L. monocytogenes* can then sometimes be relatively ineffective to prevent the consumption of unsafe food with regard to the shelf-life of foods. The exposure assessment approach described in this study could be used to establish shelf-lives consistent with a defined acceptable risk.

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1. Introduction

*Listeria monocytogenes* is a well-established food-borne pathogen and thousands of cases of listeriosis have been reported annually world-wide since 1981 (Rocourt et al., 2000). This widespread reporting is partly due to the development of surveillance systems for listeriosis in most of the industrialized countries but also to demographic changes with an increasing number of immunocompromized individuals and to changes in food habits. An increasing consumption of ready-to-eat foods having an extended shelf-life at refrigeration temperatures and supporting the growth of *L. monocytogenes* is thus observed. Several national regulatory policies include criteria for *L. monocytogenes*. These criteria are established according to the treatments received by the food during processing or before consumption (listericidal or not), the likelihood of contamination, the likelihood of multiplication during storage, distribution or use and according to the susceptibility of consumers (Codex Committee on Food Hygiene (CCFH), 2000; Huss et al., 2000; Nörrung, 2000). For ready-to-eat foods, some countries (USA and Italy) require absence of *L. monocytogenes* in 25 g of foods (“zero tolerance”), other countries (Germany, Netherlands) have a tolerable level of less than 100 *L. monocytogenes* per gram at the point of consumption and finally some countries (Canada, Denmark, France) have a tolerance of less than 100/g for some foods and a “zero tolerance” for others. This tolerance is justified by the fact that complete absence of *L. monocytogenes* may be difficult to achieve in certain foods (e.g. raw or lightly treated foods, foods recontaminated before packaging) and that it is widely assumed that a concentration of less than 100/g at the point of consumption represents a low risk for the not vulnerable consumers (International Commission on Microbiological Specifications for Foods (ICMSF), 1994).
The current French criteria for \textit{L. monocytogenes} include the following attribute sampling plans involving five samples. For foods specifically designated for highly susceptible individuals, the batches are rejected if any sample contains \textit{L. monocytogenes} in 25 g at the production or at the distribution stages. For raw or lightly treated foods or foods receiving a listericial treatment and handled before packaging, the batches are rejected at the production stage if any sample contains \textit{L. monocytogenes} in 25 g and are rejected at the distribution stage if any sample contains more than 100 \textit{L. monocytogenes} per gram. For these products, a tolerance at the production stage is applied if they comply the distribution criterion. For dairy and cooked pork meat products, the batches are rejected at the production stage if any sample contains \textit{L. monocytogenes} in 25 g and are rejected at the distribution stage if any sample contains more than 100 \textit{L. monocytogenes} per gram. The tolerance of less than 100/g at the distribution stage is allowed only if the producer is able to demonstrate that the contamination will remain below 100/g throughout the shelf-life of the food. The policy does not specify methods to use to validate this shelf-life, so two methods are currently used by food processors to validate shelf-lives for these foods towards \textit{L. monocytogenes}: (i) products (not artificially inoculated with \textit{L. monocytogenes}) are stored at +4°C during the first third of the shelf-life and at +8°C during the last two-thirds of the shelf-life and \textit{L. monocytogenes} is enumerated at the end of the shelf-life, (ii) when a natural contamination is detected in the food (i.e. presence of \textit{L. monocytogenes} in 25 g at the production stage), the manufacturer does not sell the batches and stores them in his cold store to enumerate \textit{L. monocytogenes} at the end of the shelf-life.

As establishing microbiological criteria for \textit{L. monocytogenes} is a risk management option to control the listeriosis risk for consumers, the probability of detecting non-tolerable food should ideally increase and decrease like the risk for consumers and at the same rate. The aim of this paper is then to evaluate the rate of detections for \textit{L. monocytogenes} and is used to describe the number of cells in the samples and the probability \( q = 1 - p \) for a 25 g sample to be negative is equal to \( \exp(-25c) \) where \( c \) is the \textit{L. monocytogenes} concentration (per gram). The concentration for \textit{L. monocytogenes} \( c \) is then equal to \(- (1/25) \ln(q) \). Knowing this concentration, the initial number \( n_w \) of \textit{L. monocytogenes} cells in portions of weight \( w \) (g) at the end of the production was estimated, and was described by a Poisson distribution with a mean equal to \( wc \).

2.2. Growth of \textit{L. monocytogenes}

The growth of \textit{L. monocytogenes} in the solid food portions of size \( w \) during the producer, distributor and consumer’s home storages depends on the temperature and the duration of the storage. The number of microbial cells in portions after producer and distributor storages, and at the end of the shelf-life is

\begin{align*}
\text{number} & = \text{initial concentration} \times \text{growth rate} \times \text{duration}.
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\section*{2. Exposure assessment model}

The flow chart of the exposure assessment model is shown in Fig. 1. The hypothetical analysis was done with informations easily available for food processors.

\subsection*{2.1. Initial contamination}

The prevalence of the contamination by \textit{L. monocytogenes} expressed by the proportion \( p \) of 25 g samples positive for \textit{L. monocytogenes} can be estimated from the continuous survey done by the food processor. By assuming a random distribution of \textit{L. monocytogenes} cells within the production, a Poisson distribution was used to describe the number of cells in the samples and the probability \( q = 1 - p \) for a 25 g sample to be negative is equal to \( \exp(-25c) \) where \( c \) is the \textit{L. monocytogenes} concentration (per gram). The concentration for \textit{L. monocytogenes} \( c \) is then equal to \(- (1/25) \ln(q) \). Knowing this concentration, the initial number \( n_w \) of \textit{L. monocytogenes} cells in portions of weight \( w \) (g) at the end of the production was estimated, and was described by a Poisson distribution with a mean equal to \( wc \).
Probability for a serving to contain more than 100 cfu g⁻¹

Fig. 1. Flow diagram of the exposure assessment model used to evaluate (a) the probability for a serving to be unsafe (contain more than 100 cfu g⁻¹ of *L. monocytogenes* at the end of the shelf-life), (b) the probabilities of detecting non-tolerable food using sampling plans described in French criteria, (c) the probabilities of detecting non-tolerable food using shelf-life validation protocols. Circles are used for random variables and squares indicate fixed parameters. Dotted arrows are used when two variables are linked by an equation and solid arrows are used when there is a random process between two variables. P is the probability of finding a positive sample of 25 g; C is the *L. monocytogenes* concentration at the production, C = -(1/25) ln(1 – p); n is the number of *L. monocytogenes* cells in a portion of size w, n ~ Poisson(wC); Tₘᵢₙ, Tₜₐᵢₜ, and Tₘₐₓ are minimal, optimal, and maximal temperatures for growth for *L. monocytogenes*; μₜₐᵢₜ is the maximum specific growth rate for *L. monocytogenes* at Tₜₐᵢₜ; μ₀ lag₀ is the initial value of the product μₚₐₓₙₐₙ lag for *L. monocytogenes*; T is the temperature (°C) during the storage i, Tₚₜₐᵢₜ ~ BetaPERT(2, 3, 4), T₅ₒₜₐᵢₜ ~ BetaPERT(3, 5, 7), T₅ₒₜₐᵢₜ ~ BetaPERT(2, 4, 6, 12); tᵢ is the duration (days) of the storage i, tₚₜₐᵢₜ ~ BetaPERT(2, 3, 4), t₅ₒₜₐᵢₜ ~ BetaPERT(3, 7, 11), t₅ₒₜₐᵢₜ = shelf-life – Tₙₒₜₐᵢₜ; μₜₐᵢₜ is the maximum specific growth rate for *L. monocytogenes* for the storage i, μₜ = g(Tₜ, μₜₐᵢₜ, Tₘᵢₙ, Tₜₐᵢₜ, Tₘₐₓ); lagᵢ is the lag time for *L. monocytogenes* for the storage i, lag = h(μᵢ, tᵢ₋₁, lagᵢ₋₁, μᵢ₋₁); nᵢ is the number of *L. monocytogenes* cells in a portion at the end of the storage i, nᵢ = f(tᵢ, (lagᵢ, μᵢ, nᵢ₋₁, nₘₐₓ)); dᵢ is the number of 25 g samples positive for *L. monocytogenes* among five, dᵢ ~ Binomial(5, p); d₀, d₁ and d₄ are the numbers of 10 g samples among five which contain more than 1000 cfu; (× 5) indicates that five values were generated for each iteration to simulate the contamination of five samples.

(Rosso et al., 1993):

\[
\mu_{\text{max}}(T) = \begin{cases} 
\mu_{\text{opt}} & T \leq T_{\text{min}}, \\
\mu_{\text{opt}} \frac{(T - T_{\text{opt}})(T - T_{\text{min}})(T_{\text{opt}} - T_{\text{min}})^2}{(T_{\text{opt}} - T_{\text{min}})(T_{\text{opt}} - T_{\text{min}})(T_{\text{opt}} + T_{\text{min}} - 2T)} & T_{\text{min}} < T < T_{\text{max}}, \\
0 & T \geq T_{\text{max}}.
\end{cases}
\]
where \( T_{\text{min}} \) is the temperature below which no growth occurs, \( T_{\text{opt}} \) is the temperature at which \( \mu_{\text{max}} \) is equal to its optimal value \( \mu_{\text{opt}} \) (h\(^{-1}\)), \( T_{\text{max}} \) is the temperature above which no growth occurs.

To describe the effect of temperature history on the lag time \( \text{lag}_i \) for the storage step \( i \), the following model was used (Augustin et al., 2000):

\[
\text{lag}_i = \frac{1}{\mu_i} \max(\mu_{i-1}(\text{lag}_{i-1} - t_{i-1}); 0) \quad (h \text{ model}),
\]

where \( \mu_i \) is the maximum specific growth rate for the storage step \( i \) and \( t_{i-1}, \mu_{i-1}, \text{lag}_{i-1} \) are, respectively, the duration, the maximum specific growth rate and the lag time for the previous storage step \( i-1 \).

2.3. Studied responses

2.3.1. Probability for a serving to be unsafe

The model was used to assess the numbers of \( L. \text{monocytogenes} \) in portions ingested at the end of the shelf-life. In risk assessment studies, the risk for consumers is characterized by integration of exposure assessment and hazard characterization consisting in a dose–response assessment (Lammerding and Fazil, 2000). The objective of this study was not to accurately estimate the risk for consumers but only to assess the evolution of this risk according to the prevalence of the pathogen and the shelf-life of foods. So only the probability for a portion of the food under consideration to be unsafe was considered here. The servings were regarded as unsafe when they exceeded the critical concentration of 100 \( L. \text{monocytogenes} \) per gram, i.e.
held more than $100 \cdot w$ \textit{L. monocytogenes}, where $w$ is the serving size (g).

2.3.2. Probabilities of detecting non-tolerable food

The exposure assessment model was used to assess the probabilities of detecting non-tolerable food by using sampling plans described in French criteria:

(i) $p_1$ is the probability of detecting one or more 25 g positive samples among five for a batch at the production (or at the distribution) stage, so $p_1 = p(d_1 > 0)$, where $d_1$ is the number of 25 g samples positive among five for \textit{L. monocytogenes} at the production (or at the distribution) stage. $d_1$ has a Binomial distribution with parameters 5 and $p$.

(ii) $p_2$ is the probability of detecting one or more 10 g samples among five containing more than 100 cfu per gram for a batch at the distribution stage, so $p_2 = p(d_2 > 0)$, where $d_2$ is the number of 10 g samples containing more than 100 cfu per gram among five at the end of the distribution storage.

(iii) $p_3$ is the probability of detecting one or more 10 g samples among five containing more than 100 cfu g$^{-1}$ for batches at the end of the shelf-life with storage during one-third of the shelf-life at +4°C and the remaining two-thirds at +8°C, referred to as the “validation protocol 1”. $p_3 = p(d_3 > 0)$, where $d_3$ is the number of samples of 10 g containing more than 100 cfu g$^{-1}$ among five after storages at +4°C and +8°C.
(iv) $p_4$ is the probability of detecting one or more 25 g positive samples among five for a batch at the production stage and to exceed the tolerance of 100 cfu g$^{-1}$ at the end of the shelf-life when the batch is stored by the food processor at the temperature of the cold store, referred to as “validation protocol 2”. $p_4 = p(d_1 > 0$ and $d_2 > 0)$, where $d_4$ is the number of samples of 10 g containing more than 100 cfu g$^{-1}$ among five after storage in the manufacture throughout the shelf-life.

2.4. Simulations

For simulations, the following hypothetical values or distributions were used for the input parameters of the exposure assessment model:

- $n$: analytical units of 25 g for detection tests or analytical units of 10 g for enumeration tests, and 50 g for servings ingested by the consumers;
- $T_{\text{producer}} \sim \text{BetaPERT}(2, 3, 4)$ (°C) (Vose, 2000, p. 275);
- $T_{\text{distributor}} \sim \text{BetaPERT}(3, 5, 7)$ (°C);
- $T_{\text{consumer}} \sim \text{BetaPERT}(2, 4, 6, 12)$ (°C);
- $t_{\text{producer}} \sim \text{BetaPERT}(2, 3, 4)$ (days);
- $t_{\text{distributor}} \sim \text{BetaPERT}(3, 7, 11)$ (days);
- $T_{\min} = -3^\circ\text{C}$; $T_{\text{opt}} = 37^\circ\text{C}$; $T_{\max} = 45^\circ\text{C}$ (Augustin and Carlier, 2000);
- $\mu_{\text{opt}} = 0.3$ h$^{-1}$;
- $\lambda_{\max} = 10^8$ cfu g$^{-1}$;
- $\mu_0 \text{lag}_0 = 2.1$ (Augustin and Carlier, 2000).

The size of servings and the parameters for the BetaPERT distributions are hypothetical and could be set more accurately by manufacturers knowing the actual storage regimes encountered by their products. Similarly, $\mu_{\text{opt}}$ was set for the hypothetical analysis at 0.3 h$^{-1}$ but manufacturers could use the results obtained from challenge testing with their food.

Numerical simulations were performed using independent Monte Carlo samples from the distributions of these input variables. Ten thousand iterations were performed for each value of the prevalence of the pathogen $p$ (0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.12, and 0.15) and shelf-life (20, 25, 30, 35, 40, 45, and 50 days) using the Latin Hypercube sampling with the Matlab 5.2 software (The MathWorks Inc., Natick, MA, USA) and the NAG Foundation toolbox (Numerical Algorithms Group Ltd., The MathWorks Inc., Natick, MA, USA). For each iteration the following output variables were obtained: $n_{\text{consumer}}$, $d_1$, $d_2$, $d_3$, $d_4$.

The probability for a serving to be unsafe was estimated by dividing by 10,000 the number of times that $n_{\text{consumer}}$ was greater than 5000 cfu. $p_1$ ($p_2$, $p_3$, $p_4$) was estimated by dividing by 10,000 the number of times that $d_1$ ($d_2$, $d_3$, $d_4$) was greater than 0.

3. Results

The probability for a serving to be unsafe logically increases with the prevalence of L. monocytogenes and with the shelf-life of the food (Fig. 2).

The probability $p_1$ of detecting positive 25 g samples among five for a batch at the production stage is
only related to the prevalence of *L. monocytogenes* in the food (Fig. 3) and is closed to the theoretical value of the Binomial distribution $1 - (1 - p)^5$. It is interesting to point out that this probability increases faster than the probability for a serving to be unsafe when the prevalence of the pathogen is increasing.

In this example, the probability $p_2$ of detecting samples containing more than 100 cfu g$^{-1}$ for a batch at the distribution stage was always less than 0.000. Moreover, it can be pointed out that a 10 g sample containing 1 cell of *L. monocytogenes* will contain only 636 cells with the worst storage regime, i.e. producer storage of 4 days at +4°C followed by distributor storage of 11 days at +7°C.

These results emphasize the well-known low sensitivity of the attribute sampling plans to detect batches of unsafe food. These sampling plans are only effective to prevent the sale of batches showing a high proportion of contaminated samples. They are ineffective to prevent the sale of slightly contaminated batches of foods with long shelf-lives which nevertheless, express a probability to be unsafe as high as heavily contaminated batches with short shelf-lives.

The probability $p_3$ of detecting non-tolerable food with the “validation protocol 1” depends only on the prevalence of the pathogen (Fig. 4a) because in this example, the growth of *L. monocytogenes* in the food under these storage conditions (with a shelf-life above 20 days) is so fast that the pathogen concentration in contaminated 10 g samples was always greater than approximately 4000 cfu g$^{-1}$. To obtain 10 g samples (initially contaminated with 1 cell of *L. monocytogenes*) with concentrations below 100 cfu g$^{-1}$ at the end of this validation protocol, the shelf-life must be less than 14 days. The probability of detecting non-tolerable food does then not increase with the shelf-life and is right away at its maximum value. The probability $p_3$ is equal to the probability of detecting one or more 10 g samples positive for *L. monocytogenes* among five and then only increases with increasing prevalence of the pathogen.

The probability $p_4$ of detecting non-tolerable food with the “validation protocol 2” increases with the prevalence of *L. monocytogenes* and with the shelf-life of the food (Fig. 4b). This validation protocol seems then more effective to control the listeriosis risk because, in this hypothetical example, $p_4$ is the only probability which depends on the shelf-life of the product. However, the probability of detecting non-tolerable food does not evolve with the shelf-life at the same rate as the probability for a serving to be unsafe (Fig. 5). This probability is equal to zero for shelf-lives below 30 days, afterwards it increases suddenly to reach its maximum value for shelf-lives around 40–45 days. Regarding the influence of the prevalence, the probability of detecting non-tolerable food increases approximately at the same rate as the probability for a serving to be unsafe when the prevalence of the pathogen is increasing (Fig. 3).

In this hypothetical example, the shelf-life validation protocols are then also relatively ineffective to prevent the consumption of unsafe food. On the one hand, the “validation protocol 1” can overestimate the risk and is equivalent to a “zero tolerance” criterion when the growth of *L. monocytogenes* is particularly rapid in the food. On the other hand, the “validation protocol 2” can highly underestimate the risk for medium shelf-lives and even for long shelf-lives, the maximum probability of detecting non-tolerable batches remains low (for instance, 0.08 for a prevalence of 10%).

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**Fig. 4.** Contour plots of the probabilities (a) of detecting one or more 10 g samples exceeding the tolerance of 100 cfu g$^{-1}$ among five for a batch stored at +4°C during one-third of the shelf-life and at +8°C for the remaining two-thirds, (b) of detecting *L. monocytogenes* in a batch at the production and to exceed the tolerance of 100 cfu g$^{-1}$ after storage by the producer during the shelf-life.

**Fig. 5.** Probability for a serving to contain more than 100 cfu g$^{-1}$ of *L. monocytogenes* (●), and probability of detecting *L. monocytogenes* in a batch at the production stage and to exceed the tolerance of 100 cfu g$^{-1}$ after storage by the producer during the shelf-life (▲) according to the shelf-life of the food. The probabilities were obtained for a prevalence of the pathogen equal to 10%.
4. Discussion

This hypothetical exposure assessment showed that the probabilities of detecting non-tolerable food for *L. monocytogenes* depend above all on the prevalence of the pathogen even though the probability of consumption of unsafe food by consumers depends obviously on this prevalence but also on the shelf-life of the food. It appears that the current criteria applied to manage the listeriosis risk are ineffective to prevent the risk of consumption of unsafe chilled ready-to-eat food supporting the growth of *L. monocytogenes* during the storage. These results reinforce the necessity to produce food using HACCP-based (Hazard Analysis and Critical Control Points) programs but also the necessity to properly set shelf-lives of possibly contaminated foods supporting growth of *L. monocytogenes*. The shelf-life validation protocols currently used correspond to two particular temperature regimes during storage and are not suitable. Shelf-lives of these products should, as in this hypothetical analysis, be justified by an exposure assessment approach to take into account every actual storage regimes encountered by the foods.

The exposure assessment treated in this paper is given by way of example and some assumptions have to be discussed. First, the cells of *L. monocytogenes* were assumed to be randomly distributed in the solid food and a Poisson distribution which is generally more relevant to comminuted or liquid products was used. This assumption has the consequence that contaminated servings or 10 g samples contained initially only 1 or 2 cells of *L. monocytogenes* (for the studied values of *p*, *n*0 = 1 in 84.6 to 99.0% of the contaminated servings and *n*0 = 1 in 96.8 to 99.8% of the contaminated samples). These low initial contaminations may be unrealistic and it could be more suitable in the assessment to sample an initial contamination from a known distribution for contaminated servings and samples. This approach was not used in the study because these distributions are unknown, the quantitative data available are derived from surveys done at retail and not at production stage. For this assessment, the detection test was assumed with 100% sensitivity and specificity, what is in reality seldom achieved. If a test having a sensitivity less than 100% has been used, the probability for a serving to be unsafe would be higher because of a higher actual prevalence and the sensitivity of sampling plans would be lower because some contaminated samples would not be detected. Some aspects were also deliberately neglected in this assessment to simplify the example. For a real use, the following refinements should be made:

- to use distributions for the serving size rather than a single value;
- to use distributions for the cardinal temperatures, the optimal maximum specific growth rate, the maximum bacterial concentration, and the product *μ* lag for *L. monocytogenes* to take into account the biological variability (Delignette-Muller and Rosso, 2000);
- to take into account the effect of possible injuries on lag time for *L. monocytogenes* (Bréand et al., 1999; Augustin and Carlier, 2000);
- to use a stochastic approach of microbial growth which is more suitable to model the growth of small initial cell densities by using a distribution of individual lag times rather than a population lag (Baranyi, 1998; McKellar and Knight, 2000);
- to use a distribution for the time of consumption linked to the shelf-life of the product rather than the single value of the shelf-life.

By making these refinements, the approach could be used from now on for the establishment of foods shelf-lives. As it was pointed out by Morales and McDowell (1998), this approach is now feasible but raises also a new problem: how to use the results of such assessments and what is the acceptable risk?

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References


