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# Quantitative risk assessment of *Listeria monocytogenes* in ready-to-eat foods: the FAO/WHO approach

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**Abstract**

Quantitative microbiological risk assessment is a very new and unique scientific approach able to link, for the first time, data from food (in the farm-to-fork continuum) and the various data on human disease to provide a clear estimation of the impact of contaminated food on human public health. The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have recently launched risk assessment studies of a number of pathogen–food commodity combinations (*Salmonella* in eggs and in broiler chickens, *Listeria monocytogenes* in ready-to-eat foods, *Campylobacter* in broiler chickens, *Vibrio* in seafood) to be used to lower the risk associated with these food-borne diseases and ensure fair practices in the international trade of food. The FAO/WHO *Listeria* risk assessment was undertaken in part to determine how previously developed risk assessments done at the national level could be adapted or expanded to address concerns related to *L. monocytogenes* in ready-to-eat foods at an international level. In addition, after initiation of the risk assessment, the risk assessors were asked by the Codex Committee on Food to consider three specific questions related to ready-to-eat foods in general, which are: (1) estimate the risk for consumers in different susceptible populations groups (elderly, infants, pregnant women and immunocompromised patients) relative to the general population; (2) estimate the risk for *L. monocytogenes* in foods that support growth and foods that do not support growth under specific storage and shelf-life conditions; (3) estimate the risk from *L. monocytogenes* in food when the number of organisms ranges from absence in 25 g to 1000 colonies forming units per gram or milliliter, or does not exceed specified levels at the point of consumption. To achieve these goals, new dose–response relationships and exposure assessments for ready-to-eat foods were developed. Preliminary data indicate that eliminating the higher dose levels at the time of consumption has a large impact on the number of predicted cases.


*Keywords:* *Listeria monocytogenes*; Listeriosis; Dose response; Risk assessment

## 1. Introduction

Following the implementation of the Sanitary and Phytosanitary Agreement by the World Trade Organization in 1995 [17], and the Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Consultation on Application of Risk Analysis to Food Standard Issues the same year [18], the Codex Alimentarius Commission (CAC) has intensified the development of risk assessment techniques as a priority [1]. Risk assessment, which is a part of risk analysis (with risk management and risk communication), can have a wide range of applications in food safety, such as developing broad food safety policies, sanitary measures that achieve specific food safety goals and elaborate standards for food.

The outcome of microbiological risk assessment (MRA) is to provide an estimate of the probability of illness from a pathogen in a given population. It is a structured science-based process, with four steps as defined by the CAC [1]:

- hazard identification: 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;
- hazard characterization: the qualitative and/or quanti-

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tative evaluation of the nature of the adverse health effects associated with the hazard;
- exposure assessment: the qualitative and/or quantitative evaluation of the likely intake of biological, chemical and physical agents via food as well as exposure from other sources if relevant;
- risk characterization: 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.

Microbiological risk assessment is a new approach and few studies are presently published [2]. The 32nd session of the Codex Committee on Food Hygiene (CCFH) in 1999 requested expert risk assessment advice on a number of pathogen–commodity combinations. In response, the FAO and the WHO have jointly embarked on a program of activities with the objective of providing expert advice on risk assessment of microbiological hazards in food [18–22]. Risk assessment of a number of pathogen–food commodity combinations selected from the priority list developed by CCFH are under development by FAO and WHO (*Salmonella* in eggs and in broiler chickens, *Listeria monocytogenes* in ready-to-eat (RTE) foods, *Campylobacter* in broiler chickens, *Vibrio* in seafood).

## 2. *L. monocytogenes* risk assessment

### 2.1. Scope and objectives of the FAO/WHO L. monocytogenes risk assessment

The overall goal of the risk assessments organized by FAO/WHO is to lower the risk of the disease under study.

The *Listeria* risk assessment (Members of the *Listeria* Drafting Group: R. Buchanan, USA; R. Lindqvist, Sweden; M. Smith, Canada; E. Todd, Canada/USA; T. Ross, Australia; R.C. Whiting, USA) was undertaken in part to determine how previously developed risk assessments done at the national level could be adapted or expanded to address concerns related to *L. monocytogenes* in RTE foods at an international level. In addition, after initiation of the risk assessment, the risk assessors were asked by the 33rd session of the CCFH, through FAO and WHO, to consider three specific questions related to RTE foods in general. These questions are:

1. estimate the risk for consumers in different susceptible populations groups (elderly, infants, pregnant women and immunocompromised patients) relative to the general population;
2. estimate the risk for *L. monocytogenes* in foods that support growth and foods that do not support growth under specific storage and shelf-life conditions;
3. estimate the risk from *L. monocytogenes* in food when the number of organisms ranges from absence in 25 g to 1000 colonies forming units (CFU) per gram or milliliter, or does not exceed specified levels at the point of consumption.

### 2.2. Hazard identification

Listeriosis is one of the most severe food-borne infections (meningitis, septicemia, abortion), with low morbidity (annual incidence rate ranging from 2 to 10 cases per million population) but high lethality (30%), and a high predilection for people with impaired T-cell immunity (pregnant women and neonates, immunocompromised patients, the elderly). Listeriosis is mainly reported from industrialized countries, and the reported incidences in Africa, Asia and South America are low. Whether this reflects different consumption patterns and dietary habits, different host susceptibility, different food processing and storage technologies or lack of awareness, reporting or laboratory facilities is not known. Most cases of human listeriosis appear to be sporadic, although a proportion of these sporadic cases may be unrecognized, common-source clusters. The source and route of infection of the great majority of these cases remain unknown, although food-borne transmission is demonstrated in some cases. Epidemiological investigations of all large outbreaks that have occurred since 1981 demonstrate that nearly all kinds of RTE foods can transmit the disease and that the outbreaks were associated with industrially manufactured foods [3].

In many respects, *L. monocytogenes* differs from most known food-borne pathogens in that it is ubiquitous, resistant to diverse environmental conditions (despite being asporogenous) including low pH and high NaCl concentrations, and is microaerophilic and psychrophilic. The various ways the bacterium can enter a plant, its tenacity in the industrial environment, its ability to grow at very low temperatures and to survive in food for prolonged periods under adverse conditions have made this bacterium one of the hottest topics for the food industry during the last two decades.

### 2.3. Hazard characterization

Dose–response relationships associated with *L. monocytogenes*, and more generally with food-borne pathogens, derive from various data sources which all offer strengths and limitations to variable degrees in the understanding of the pathogen–host food matrix interactions [4]. Main data sources used for *L. monocytogenes* include:

- food-borne outbreaks: invasive listeriosis (outbreak associated with Mexican-style cheese in 1985 in USA, associated with butter in Finland in 1999), and gastroenteritis (outbreak associated with chocolate milk in USA in 1993 and with corn salad in Italy in 1997) [5];
- data from surveillance of invasive listeriosis combined with data from *Listeria* in food [6,7];

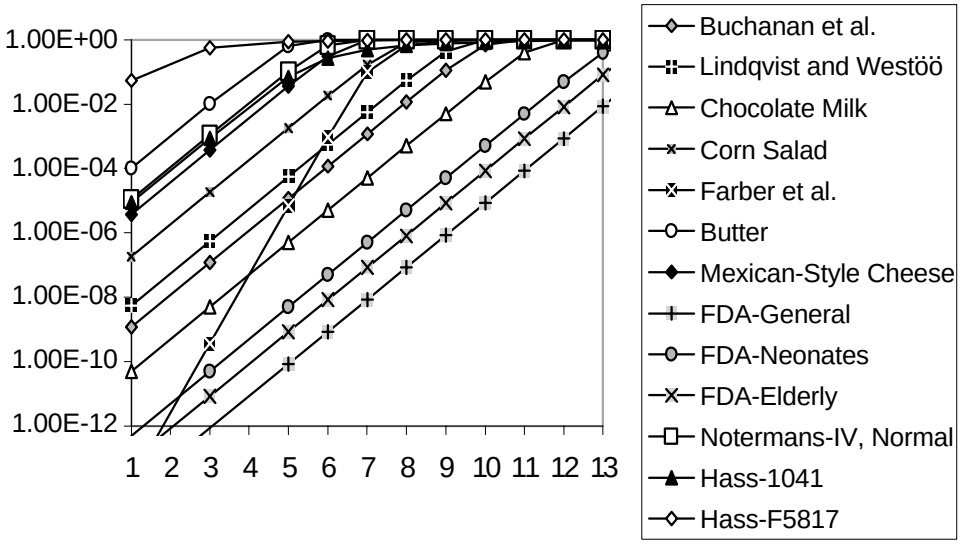


Fig. 1. Examples of dose–response models [11].

- relative susceptibility of different subpopulations combined with data of an exposure assessment [8];
- laboratory animals models [5,9,10].

Using these sources of data, biological endpoints (invasive listeriosis, gastroenteritis, mortality), various hosts (different susceptible human populations, mouse experiments) and different mathematical models (exponential, Weibull-Gamma, Beta-Poisson, combination of multiple mathematical models), a number of dose–response relationships have recently been described (Fig. 1) [11]. All models have assumed that, in theory, a single cell has the potential to cause disease. Estimated $r$ values (probability of a single cell causing the biological end-point under study) range from $1\times10^{-5}$ to $8\times10^{-16}$ [5,8,11].

Each of these models has specific characteristics and limitations. These models are based on different endpoints and different types of data. However, no single model met the needs of the FAO/WHO risk assessment. An alternative model, more appropriate for questions posed by CCFH, was therefore developed by applying the exponential model to data from human listeriosis surveillance in the USA and data of the FDA/USDA-FSIS exposure assessment [8]. This approach implicitly considered the variability in virulence among *L. monocytogenes* strains, the variability in host susceptibility (the normal population and the various subpopulations more at risk of listeriosis) and the variability in foods able to transmit the disease.

Additional models were developed by the Drafting Group to answer CCFH question no. 1 regarding the evaluation of the risk from *L. monocytogenes* to susceptible population groups compared to the general population. They were derived from relative susceptibilities calculated from epidemiological data on listeriosis in France. Calculated $r$ values showed a wide range, from $1.4\times10^{-10}$ to $10^{-15}$, patients with recent organ transplantation or suffering from AIDS being the most susceptible subpopulations (Table 1).

Present limitations in dose–response models reflect the need for further data such as virulence heterogeneity of *L. monocytogenes*, impact of the food matrix on the ability of *L. monocytogenes* to cause disease, epidemiological data related to outbreaks and sporadic cases of listeriosis and better estimates of the actual populations at increased risk of invasive listeriosis [11]. Dealing with dose response implies therefore a careful evaluation of the strengths and limitations of the data selected for these estimations since they are critical to the outcome. Such evaluations will also make it possible to establish the uncertainty associated with dose–response models that are developed from different data sets and models.

### 2.4. Exposure assessment

Exposure assessment is the estimation of how likely it is

Table 1
Relative susceptibility and $r$ value for immunocompromised and non-immunocompromised populations [8]

| Population | Relative susceptibility | $r$ value[a] |
|---|---|---|
| France | | |
| Organ transplant | 2584 | $1.4\times10^{-10}$ |
| AIDS | 865 | $4.6\times10^{-11}$ |
| Dialysis | 476 | $2.5\times10^{-11}$ |
| Cancer, bladder | 112 | $6.0\times10^{-12}$ |
| Cancer, gynecological | 66 | $3.5\times10^{-12}$ |
| Elderly (over 65 years old) | 7.5 | $4.0\times10^{-13}$ |
| Non-immunocompromised | 1 | – |
| USA | | |
| Elderly (over 60 years old) | 1.6 | $8.4\times10^{-12}$ |
| Perinatal | 839 | $4.5\times10^{-11}$ |
| Non-immunocompromised | 1 | – |

[a] $r$ value $5.3\times10^{-11}$ from maximum dose at $10^{8.5}$ used for reference population.

that an individual or a population will be exposed to a pathogen via food and what numbers of the pathogen are likely to be ingested. Three types of information are usually necessary to do this assessment: information on the prevalence and concentration of the pathogen in raw ingredients, information on the effects that food processing, distribution, handling and preparation steps have on the pathogen and information on consumption patterns. This involves collecting sufficient, relevant and accurate data, generating estimates, creating models and/or making assumptions to describe the prevalence and the concentration of the pathogen at each step of the farm-to-fork continuum.

Since the most relevant food related to *L. monocytogenes* are RTE foods, stored a long time at refrigeration temperatures, previous studies have focused on those foods [12–16]. Reflecting the fact that *Listeria* is a ubiquitous organism, none of these studies fully encompasses a farm-to-fork approach. The most extensive study assessed exposure with *L. monocytogenes* growth modeled only from retail to consumption.

Considering the available resources and time constraints placed on the Drafting Group, it was impossible to consider all RTE foods that could be contaminated with *L. monocytogenes* in the FAO/WHO risk assessment. Accordingly, it was decided to limit the risk assessments to a finite range of RTE foods that have been selected to represent various classes of product characteristics, in order to determine whether the risk of these foods serving as a vehicle for human food-borne listeriosis can be estimated. It was also decided to limit the study to foods at retail and their subsequent public health impact at the time of consumption. This was done for two reasons. Firstly, it was sufficient to address the charge provided by the CCFH within the time frame and resources available to the risk assessors. Secondly, most of the exposure data for *L. monocytogenes* that are currently available relate to the frequency and extent of contamination at the retail level. Therefore, this risk assessment does not evaluate the risk associated with different means of manufacturing these products. However, it does consider several post-retail factors that could influence the consumer risk of acquiring food-borne listeriosis such as temperature and duration of refrigerated storage.

Four different RTE foods are presently under study – fluid milk, ice cream, semi-fermented meat products and smoked fish – which were selected to provide examples of how microbiological risk assessment techniques can be used to answer food safety questions at an international level. Results will be available in the near future.

Present data on exposure assessment of *L. monocytogenes* in RTE foods clearly indicate significant gaps in the data required, especially for a farm-to-fork approach. More research is needed in a number of areas such as: prevalence of *L. monocytogenes* in agricultural and aquatic environments, prevalence of *L. monocytogenes* in primary and secondary production, variability of *L. monocytogenes* virulence, evaluation of predictive models for *L. monocytogenes* in specific products, identification of sources and levels of contamination and recontamination, prevalence and concentration of *L. monocytogenes* in finished packages of RTE foods, retail and consumer handling practices and specific RTE product consumption data, especially for populations at higher risk [11].

### 2.5. Risk characterization

Risk characterization is the estimation of the probability of occurrence and severity of adverse health effects in a given population based on hazard characterization and exposure assessment. Risk characterization of *L. monocytogenes* in RTE food has been poorly investigated until now [12]. Data on risk characterization for examples of RTE foods, either lightly or highly processed and either allowing or not allowing *Listeria* growth, are under development by the Drafting Group.

Preliminary data for CCFH question no. 3 on the risk associated with different levels of *L. monocytogenes* in foods were generated using the dose–response model established for the FAO/WHO risk assessment and the FDA/USDA-FSIS exposure assessment during an FAO/WHO expert consultation [8]. These data clearly demonstrate that eliminating the higher dose levels at the time of consumption has a large impact on the number of predicted cases (Table 2). However, it is important to note that this is based on 100% compliance. It is also important to emphasize that these data consider cell numbers at the time of consumption. Consideration of cell numbers at the time of retail would have to be corrected to take into account the potential increases in *L. monocytogenes* that would occur as a result of growth in those foods that will support *L. monocytogenes* replication.

## 3. Conclusions

Microbiological risk assessment is a unique scientific

Table 2
Number of predicted cases if various criteria for CFU/serving could be realized at 100% effectiveness [8]

| Maximum log dose at consumption (log CFU/serving) | Number of predicted cases |
|---|---|
| Baseline distribution[a] | 2130 |
| 4.5 | 24.9 |
| 3.5 | 5.3 |
| 2.5 | 1.1 |
| 1.5 | 0.2 |
| 0.5 | 0.06 |
| −0.5 | 0.02 |
| −1.5 | 0.01 |

[a]This depicts the current number of predicted cases based on a previously described distribution of *L. monocytogenes* [8].

approach able to link, for the first time, data from food (in the farm-to-fork continuum) and the various data on human disease to provide a clear estimation of the impact of contaminated food on human public health. It is also the most powerful tool available today to clearly assess the efficacy of each possible mitigation strategy. The development and use of this tool encourages international collaboration and provides a transparent system for comparison of food-borne risk as well as mitigation potential. Microbiological risk assessment therefore provides risk managers with clear information to lower the risk associated with food-borne pathogens at national and international levels.

The present data on *Listeria* risk assessment, as well as those available for other pathogen–food commodity combinations, indicate that the modeling approach is applicable to quantitative MRA, and more especially to *L. monocytogenes*. According to present knowledge, a FAO/WHO expert consultation concluded that dose–response models could be presently applied to different countries. However, models would probably gain in international applicability through use of input data from different regions of the world. Similarly, the models developed for exposure assessment are applicable by others when computer programs are made available [8].

This exercise in conducting risk assessment at the international level identified crucial limitations due to gaps in scientific knowledge and underlined the need for data to be acquired from all regions. It also demonstrated that MRA is a multidisciplinary approach involving clinical veterinarians, food technologists and microbiologists, experts in food consumption, modelers, as well as clinicians and epidemiologists. More research on pathogens in foods, on pathogen characteristics and on human food-borne diseases based on international multidisciplinary cooperation is strongly needed, and MRA should be used to guide such research. The scientific community has a crucial role to play in public health by providing risk assessors with the necessary data so that MRA can be regularly refined.

More information can be found at the following internet addresses:

- http://www.who.int/fsf/mbriskassess/index.htm
- http://www.fao.org/WAICENT/FAOINFO/ECONOMIC/ESN/pagerisk/riskpage.htm

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