

APPROVED: 7 September 2020

doi: 10.2903/j.efsa.2020.e181103

Training in tools to develop Quantitative Risk Assessment using Spanish ready-to-eat food examples

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Abstract

Unsafe food poses global health threats, potentially endangering consumers. The great majority of people will experience a food-borne disease at some point in their lives. Ready-to-eat (RTE) food is the one intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce the concentration of pathogenic microorganisms. Prepared foods are often complex and may contain multiple components that make them vulnerable for growth of pathogenic microorganisms. Among all the pathogenic microorganisms that may be present in RTE foods, *Listeria monocytogenes* is of special interest because it is the causative agent of listeriosis and it has the ability to survive and replicate at refrigeration and low pH conditions. We performed a quantitative microbial risk assessment (QMRA) in RTE dry-fermented sausage to measure the risk of listeriosis associated to the consumption of this product. The starting point of our investigation was the storage at the factory, after the end-product was produced and before distribution to retail. The stochastic model was implemented in MicroHibro, an online tool for QMRA. Because *L. monocytogenes* concentration and prevalence can vary greatly between different studies and different types of fermented sausages, we tested different scenarios to show the importance of low prevalence and concentration of the pathogen at the final product. Our results show that the risk estimates are very sensitive to the modelling hypotheses used to describe this process. Therefore, the development of accurate probabilistic models describing the initial concentration of *L. monocytogenes* shall largely reduce the uncertainty associated to the QMRA of listeriosis in this type of product.

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Keywords: *Listeria monocytogenes*, risk assessment, RTE meat, QMRA, food safety

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Acknowledgements: This report is funded by EFSA as part of the EU-FORA programme.

Suggested citation: Departamento de Ingeniería Agronómica. ETSIA-Universidad Politécnica de Cartagena, Paseo Alfonso XIII, 48, 30203. Cartagena, Región de Murcia. Spain, Georgalis L, Garre A and Fernandez Escamez PS, 2020. Training in tools to develop Quantitative Risk Assessment using Spanish ready-to-eat food examples. *EFSA Journal* 2020;18(S1):e181103, 10 pp. <https://doi.org/10.2903/j.efsa.2020.e181103>

ISSN: 1831-4732

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The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.



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1. Introduction

Unsafe food poses global health threats, potentially endangering consumers. As a result, the great majority of people will experience a food-borne disease at some point in their lives. This highlights the importance of making sure the food we eat is safe from potentially harmful bacteria, parasites, viruses, toxins and/or chemicals (World Food Summit, 1996). Food safety remains as one of the main concerns in every Member State of the European Union, including Spain. In 2018, EU Member States reported 5,146 food-borne outbreaks affecting 48,365 people (EFSA & ECDC, 2019). A food-borne disease outbreak is defined as an incident during which at least two people suffer the same illness after ingesting the same contaminated food or drink. Food-borne disease has long represented a considerable burden to public health and continues to challenge health systems worldwide. Although anyone may contract a food-borne disease, vulnerable populations such as small children, elderly people, pregnant women, immunocompromised people and those living in poverty or who are food insecure are particularly vulnerable (WHO, 2017). The global scale of the supply chain in modern food production has reduced the cost and increased the variety of food available, but this integration of the food supply enable food-borne pathogens and toxins to infect and poison large numbers of consumers (Ercsey-Ravasz et al., 2012; Garre et al., 2019a).

Ready-to-eat (RTE) food is food intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to an acceptable level microorganisms of concern (2019/229 of 7 February 2019 amending regulation (EC) No 2073/2005). The lack of a cooking step prior to consumption makes this type of product especially susceptible for being a source of food-borne disease. *Listeria monocytogenes* is a ubiquitous organism that is widely distributed in the environment. It can contaminate foods and cause gastroenteritis (a mild, non-invasive illness) or listeriosis (a severe, invasive illness) (World Health Organization, & Food and Agriculture Organization of the United Nations, 2004). The most important characteristic of listeriosis is the relatively high mortality rate compared to illnesses caused by most other food-borne pathogens (15.6% compared to < 1% for *Salmonella* or *E. coli* O157) (EFSA & ECDC, 2019). Furthermore, mortality may be underestimated among elderly in nursing homes and care facilities (EFSA BIOHAZ Panel, 2018), while having an underlying disease is a risk factor for increased mortality (Goulet et al., 2012). In 2018, 28 Member States reported, 2,549 confirmed invasive human cases of listeriosis with an EU notification rate of 0.47 cases per 100,000 population (EFSA & ECDC, 2019). The capacity of *L. monocytogenes* to grow under extreme conditions such as low temperature, low pH and high salt is among the most important factors affecting the risk of human listeriosis associated with consumption of RTE foods. Growth may occur both in foods and in the environment, while other elements affecting *L. monocytogenes* growth include water activity, concentration of antimicrobials, storage temperature and time (EFSA, 2018). On 16 August 2019, Regional Health Authorities in Andalusia, Spain, reported an outbreak of listeriosis, caused by the bacteria *L. monocytogenes*, associated with the consumption of a chilled roasted pork meat product manufactured in Spain and sold under the brand name 'La Mechá'. A total of 222 confirmed cases linked to this outbreak were reported, including three deaths among elderly persons who were ill with listeriosis at the time of death (Ministerio de Sanidad, Consumo y Bienestar Social, 2019).

Quantitative microbial risk assessment (QMRA) is a mathematical modelling approach used to estimate the risk of infection when a population is exposed to microorganisms. The process of QMRA involves identifying and characterising the hazards, assessing exposure, and characterising the risk (European Commission Scientific Committee for Food, 1997). QMRA is based on a quantitative description of the microbial response to the different conditions encountered during each step of the field-to-fork chain of the product based on mathematical models. However, this system is inherently stochastic. For that reason, it is essential to include (and ideally separate) uncertainty and variability in the analysis (Benford et al., 2018). In this context, variability refers to sources of variation that are inherent to the system (e.g. biological differences between microbial cells), whereas uncertainty is related to lack or imprecise knowledge (Nauta, 2000; Koutsoumanis and Aspidou, 2017). This can be accomplished by a stochastic modelling approach where the relevant kinetic parameters are described using probability distributions (van Boekel, 2020; Garre et al., 2020). Numerous studies have pointed out that the prediction of the outbreak size may depend on the way that uncertainty and variability are separated, and that a major outbreak may be overlooked if the distinction between uncertainty and variability is neglected (Nauta, 2000; Benford et al., 2018; Garre et al., 2020).

2. Description of work programme

2.1. Aims

As part of the EU-FORA fellowship, the focus of this study was for the fellow to be involved in all the activities required for the risk assessment of RTE foods, from data analysis to modelling alternatives, establishing different scenarios and performing a QMRA. The final outcome will be the estimation of the risk based on different scenarios for susceptible groups: persons over the range of 60–65, infants, pregnant women and immunocompromised patients. This objective will focus on establishing the health risks on the basis of conditions included in the study using existing web-based tools (MicroHibro) and the data and models already available in the scientific literature. This will allow the establishment of risk interpretation of the impact of variability and uncertainty on a QMRA. The fellow will gain skills related to the interpretation and communication of the outcome of a QMRA using software tools broadly used by the community.

2.2. Activities/methods

As part of the fellowship, the priority of the hosting site was to provide the fellow with the basic theoretical background required to perform a QMRA. The fellow joined a working team with proved expertise in the use of risk assessment tools and received training on specific topics such as:

- Handling of available databases (EFSA, FAO, the group's database for microbial inactivation, etc.);
- Optimal Experimental Design (including the bioOED software, developed in the group) applied to inactivation experiments;
- Growth and inactivation modelling (such as Combase or Bioinactivation, the latter developed in the group);
- Statistical analysis using Monte Carlo and Bayesian methods and risk ranking methodologies;
- Software tools specific for risk assessment (e.g. MicroHibro and FDA-iRISK);
- Separation between variability and uncertainty, the quantification of these terms and the incorporation in predictions from the point of view of experimental design and statistical analysis.

2.2.1. Laboratory experience gained

In order to better understand the empirical methods required to gather the data needed to build predictive models for QMRA, part of the training included the characterisation of the response of *L. monocytogenes* to thermal inactivation treatments at both constant and varying temperatures. Experiments were performed using *L. monocytogenes* CECT 4032, supplied by the Spanish Type Culture Collection.

Training at bacterial strains and culture conditions

A freeze-dried sample was transferred to 10 mL of tryptic soy broth (TSB) (Scharlab Chemie S.A., Barcelona Spain) for rehydration during 30 min. Then, 5 mL of culture were inoculated in 500 mL of TSB and incubated for 21 h at 37°C with constant stirring at 200 rpm. At that time, cells reached stationary growth phase. Subsamples of the culture were frozen with glycerol (1:1) at –20°C and stored until use. To perform experiments, a frozen sample was inoculated in a tryptic soy agar (TSA) plate and incubated at 37°C for 24 h. Then, a colony was selected, inoculated in TSB and grown with constant stirring overnight until cells reached the stationary growth phase.

Thermal treatments and enumeration of survivors

Thermal treatments were carried out using a Mastia thermoresistometer (Conesa et al., 2009). This device allows to perform thermal treatments in liquid media with a temperature profile that can be programmed within the maximum heating and cooling rates of the equipment (40°C/min). The vessel of the thermoresistometer is constantly stirred during the treatment, ensuring a homogeneous temperature distribution. Before starting the treatment, the vessel was filled with 400 mL of the heating medium. Sterile TSB was used as heating media.

Isothermal experiments were performed at 55, 57.5, 60, 62.5 and 65°C. For each treatment, the thermoresistometer was programmed at constant temperature. Once the temperature in the vessel

was stable, a 0.2 mL volume of the microorganism suspension was inoculated. For non-isothermal conditions, eight different temperature profiles were tested, that can be grouped in two different categories: monophasic profiles with constant heating rate, and biphasic profiles with a holding phase after the initial heating rate. Viable counts were determined following the same procedure for both isothermal and dynamic profiles. Samples with a volume of 3 mL were taken at preset times and collected in sterile test tubes, which were immediately cooled in ice. They were based on duplicate counts from appropriate dilutions, of 1 mL aliquots, that were plated and mass homogenised in TSA. Plates were incubated at 37°C for 24 h and then counted (Garre et al., 2019b).

2.2.2. Mathematical modelling

The fellow was involved in the development and application of stochastic mathematical modelling for QMRA. We used MicroHibro (González et al., 2019), a web tool for QMRA based on stochastic models. This type of model uses a bottom-up (or forward) approach considering the steps of initial microbial concentration (including prevalence), growth, transfer, reduction and dose–response.

L. monocytogenes has been studied extensively during the past decade, regarding its potential to produce food-borne illnesses and it is being closely monitored by health authorities worldwide (Buchanan et al., 2017; Churchill et al., 2019; Garre et al., 2019b). For this reason, we performed a literature review, in order to find and use the most appropriate data for the QMRA.

The prevalence of *L. monocytogenes* in cured meats has been studied in different Spanish regions with substantial variation between the study findings. In Navarra, for example, they found a prevalence of 6.7% in cured meats ('salami', 'chorizo', 'salchichon'), while prevalence dropped to 2.7% in vacuum-packed deli meat products and increased to 8.5% in opened deli meat products (Vitas and Garcia-Jalon, 2004). A study from Catalonia showed that 1/9 (11.1%) of pork luncheon meat, 11/65 (16.9%) of cured dried pork sausage and 3/24 (12.5%) of cooked ham were *L. monocytogenes* positive (Cabedo et al., 2008). In Zaragoza, 6 out of 35 RTE cooked meat products (17.14%), 21 out of 57 RTE raw-cured meat products (36.84%), and 9 out of 37 RTE dry-cured, salted meat products (24.32%) were *L. monocytogenes* positive (Gomez et al., 2015).

The starting point of our exercise was the storage at the factory, after the final product was ready and before distribution to retail (Figure 1). We decided to use the data available from a recent study coming from Córdoba, Spain (Possas et al., 2019) which built a probabilistic model to predict the fate of *L. monocytogenes* in Spanish chorizo sausage from mixing of raw materials up to consumption (Table 1).



Figure 1: Flow chart of the product pathway and distribution chain

The data used to build the QMRA model consists of the following main parameters: 1) the prevalence of the pathogen at retail level; 2) the concentration of the pathogen at retail level; 3) the growth of the pathogen during storage; 4) the serving size; 5) the total number of annual servings; 6) the population of interest; and 7) the dose–response based on an exponential model. Additional data information were also extracted from EFSA's Scientific Opinion on the development of a risk ranking toolbox (EFSA BIOHAZ Panel, 2015).

Table 1: Overview of the probabilistic model input variables

Model phase	Input variable	Description	Distribution/ model/value	Unit	References
Initial concentration	N_0	Initial concentration	Normal (2.42, 1.016)	log CFU/g	EFSA BIOHAZ Panel (2015)
	P	Prevalence	7.5	%	Possas et al. (2019)
	L_g	Lag time	0	–	Possas et al. (2019)
	W	Sausage weight	100	Grams	–
	G	Growth at storage	0.425	log ₁₀ CFU/g	EFSA BIOHAZ Panel (2015)
Storage at factory	t_{st}	Storage at the factory duration	Uniform (0, 36)	h	Nauta et al. (2003)
	T_{st}	Storage temperature at the factory	5	°C	Marcos et al. (2013)
Retail	t_R	Storage time at retailing	Uniform (2, 6)	h	Possas et al. (2019)
	T_R	Temperature at retailing	Normal distribution (3.7, 1.78)	°C	Possas et al. (2019)
Transport from retail to home	t_{TR}	Transport to home time	Uniform (0.25, 2)	h	Possas et al. (2019)
	T_{TR}	Transport to home temperature	Triangular distribution (10; 4; 25)	°C	Nauta et al. (2003)
Consumption	t_H	Household storage time	Normal distribution (4.3, 2.6)	h	Nauta et al. (2003)
	T_H	Household temperature	Normal (6.62, 2.56)	°C	Carrasco et al. (2007)
	S_z	Serving size	Normal distribution (50, 5)	Grams	–
	D_R	Dose–response	Equation: $1 - \exp(-r \times \text{pow}(10, \text{dose}) \times \text{serving})$	–	World Health Organization & Food and Agriculture Organization of the United Nations (2004)
Population	Population of interest	Spanish population with an estimate that 10% is considered highly susceptible	47.000.000	Persons Persons	–
	Simulations	Number of model simulations	100	–	–

CFU: colony forming unit.

Effect of prevalence and initial concentration of *L. monocytogenes*

As evidenced from the preliminary review of the scientific literature, *L. monocytogenes* concentration and prevalence can vary greatly between different studies and different types of fermented sausages. Therefore, in order to assess how different modelling hypotheses regarding the initial concentration levels affect the risk estimates, a scenario analysis where different distributions were compared was applied. Originally, we defined a normal distribution (2.42, 1.016) as in the study by (EFSA BIOHAZ Panel, 2015) As an alternative scenario, we used a uniform distribution of the initial concentration (Uniform (0,3)) as in the study by (Possas et al., 2019).

3. Conclusions

3.1. Conclusions from the probabilistic assessments

The contamination of RTE meat products with *L. monocytogenes* is a major concern for the food industry. Mathematical modelling simplifies processes occurring in the physical world through a series of hypotheses. The aim of this project for the fellow was to develop technical skills relevant in risk assessment stages: i) hazard identification, ii) hazard characterisation, iii) exposure assessment and iv) risk characterisation.

Our model predicted a risk of 0.002363 illness per serving (min = 3.96E-07, max = 0.085165, SD = 0.01) and a total number of 416 cases, in the Spanish population (using input parameters of Table 1). This number is higher than the 372 cases of listeriosis that was recorded in Spain for the year 2018 (EFSA & ECDC, 2019). Overestimation of the predicted cases may be due to the different resources, databases and distributions of the input variables that were used for the estimation of listeria QMRA in this report.

An example of how much the predicted risk can be influenced by the type of input variables and distributions, we analysed the sensitivity of the number of cases to the hypotheses used to describe the initial concentration in the product. When we applied a Uniform distribution with a range from 0 to 3 log CFU/g, the predicted risk of cases per serving decreased to 0.000111 (min = 7.26307E-07, max = 0.000772, SD = 0.00018) with only 20 predicted cases. As expected, the prevalence of the final product at the retail level plays a significant role in the expected risk of infection. Apart from the original prevalence (Table 1), seven more prevalence concentrations of *L. monocytogenes* were tested based on the findings of different studies. The first group of prevalence tested less than 7.5%, namely 1.5%, 3% and 5% showed the same risk per serving (0.00236 illness/serving) with 83, 167 and 278 predicted cases, respectively. On the other hand, prevalence concentration of 10%, 17%, 24.3% and 36.84% increased the predicted number of cases at 555, 944, 1,349 and 2,045, respectively.

Therefore, the hypotheses used to describe the initial concentration in the product can largely influence the predicted risk. This is especially relevant considering the large variability in the prevalence levels estimated in different studies published in the scientific literature, which can range from 2.7% to 38.84%. The uncertainty about the outbreak size increases with increasing uncertainty in the input distributions (Nauta, 2000). Hence, the development of more accurate probabilistic models to describe the initial concentration of *L. monocytogenes* in this type of product shall greatly reduce the uncertainty associated to the risk estimates.

3.2. Scientific activities during fellowship

During the fellowship, the fellow had the opportunity to participate to various scientific activities. These included attending various conferences/seminars/webinars:

- IV Jornada Cátedra AgroBank. *Technological challenges in the cultivation and post-harvest of fruit and vegetables*. Universidad Politécnica de Cartagena, Spain, 12 November 2019.
- Escuela Técnica Superior de Ingeniería Agronómica. *The use of mathematical models and statistical tools to navigate an ocean of data*. Universidad Politécnica de Cartagena, Spain, 18 November 2019.
- Escuela Técnica Superior de Ingeniería Agronómica. *Concepts regarding food shelf life (definitions, legislation, date marking, quality and safety issues, sensory vs consumer panels and finally shelf life estimation methods)*. Universidad Politécnica de Cartagena, Spain, 4 December 2019.
- Escuela Técnica Superior de Ingeniería Agronómica. *Modelling approaches to estimate shelf life for a specific food product based on a set of experimental data*. Universidad Politécnica de Cartagena, Spain, 5 December 2019.
- Misión Posible: *De Horizonte 2020 a Horizonte Europa*. Universidad Politécnica de Cartagena Spain, 23 January 2020.
- Presentación del Programa PRIMA: *The Partnership for Research and Innovation in the Mediterranean Area*, Universidad Politécnica de Cartagena, Spain, February 13 2020.
- VectorNet with ECDC & EFSA collaboration: *Webinar 1 - VectorNet Maps: What are they and how to use them*. Online course 18 February 2020.

- GRUPOGAM. *Prevention and Control of Contamination by Listeria in Food*, Universidad de Murcia, Spain, 20 February 2020.
- Escuela Técnica Superior de Ingeniería Agronómica. *Modeling and control of irrigation and fertilizers in greenhouses*. University of Almería, February 28, 2020.
- Escuela Técnica Superior de Ingeniería Agronómica. *International Seminar: Technological advances in the post-harvest fruit and vegetable*. Universidad Politécnica de Cartagena Spain, 11 March 2020.
- EFSA. Webinar: *Rapid Assessment of Contaminant Exposure (RACE) tool*, 12 March 2020.
- One Health EJP: *Annual Scientific Meeting 2020*, Online Congress, Prague, 27–29 May 2020.
- EFSA Online Course: *Parma Summer School 2020 'One Health'*. 9–10 June 2020.

3.3. Conclusions from the participation in the fellowship programme

The main focus of the work programme was the development and application of a quantitative microbial risk assessment model in order to estimate the public health risk for listeriosis following consumption of Spanish ready to eat fermented sausage. The work plan provided training and knowledge on all the steps and tools required to perform a QMRA, taking as an example food/pathogen combinations relevant for Spanish ready-to-eat products. The fellow was involved in all the activities of the risk assessment process, from data analysis to modelling alternatives, establishing different scenarios and performing a QMRA. He had a very significant contribution during all the steps of the programme.

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Abbreviations

EU-FORA	European Union Food Risk Assessment
FAO	Food and Agriculture Organization
QMRA	quantitative microbiological risk assessment
RTE food	ready-to-eat food
TSA	tryptic soy agar
TSB	tryptic soy broth