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Training in tools to develop quantitative microbial risk assessment along the food chain of Spanish products

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Abstract

Food safety is a widespread challenge. Every year it is estimated that almost 1 in 10 people in the world fall ill after eating contaminated food resulting in over 400,000 deaths. The risk of outbreaks is higher when consuming ready-to-eat (RTE) products because they are eaten without a further cooking process that could inactivate pathogenic microorganisms. Hence, food processing is essential to increase the safety of RTE products. Microbiological risk assessment (MRA) integrates food science, microbiology and data science to provide a comprehensive understanding of the safety of the food system. MRA provides qualitative and/or quantitative information to decision makers, which might promote the adoption of better food practices. In this contest, this project aims to study and implement tools for quantitative microbial risk assessment (QMRA) of food products along the food chain. A common RTE product (cured ham) from Spain was used as a case study. Following, the exposure assessment model was implemented using mathematical models and statistical software to describe the microbial behaviour along the food chain. The study presents the possibility to identify the risk exposure in different scenarios (e.g. growth during different storage conditions, inactivation induced by traditional or innovative decontamination techniques), showing the flexibility of the predictive tools developed.

Keywords: QMRA, ready-to-eat, *Listeria monocytogenes*, supercritical CO_2, low-temperature pasteurisation

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1. Introduction

1.1. Food safety in ready-to-eat meat products

Food safety is a widespread challenge. Every year almost 1 in 10 people in the world fall ill after eating contaminated food resulting in over 400,000 deaths (WHO). The risks of contamination are relevant in every step from farm to fork and require prevention and control throughout the whole food chain. The consumption of food from animal origin is associated with a high risk for the population. Indeed, infectious disease can be spread between animals and people by pathogenic microorganisms such as bacteria, viruses and parasites. Specifically, meat contamination can occur at the farm (e.g. parasites that infect food-producing animals), at slaughter (e.g. contamination by intestinal contents), during processing (e.g. cross-contamination by handling/surface/food) and preparation (e.g. improper use of safe handling practices). In 2020, the consumption of foods of animal origin was responsible for 65.7% of outbreaks in the EU and campylobacteriosis and salmonellosis were the first and second most reported zoonoses in humans in 2020 (EFSA and ECDC, 2021), followed by *Yersiniosis*, Shiga toxin-producing *Escherichia coli* (STEC) and *Listeria monocytogenes* infections. Among these pathogens, *L. monocytogenes*, a Gram-positive and non-spore-forming bacterium that can grow under anaerobic and aerobic conditions, has the highest reported deaths, with a 13% of fatality rate in the EU in 2020. Listeriosis is a serious infection caused by *L. monocytogenes* which are transmitted to humans by contaminated food (99% of cases). Listeriosis occurs in both invasive (maternal-neonatal and non-maternal-neonatal) and non-invasive (gastroenteric) forms. Invasive listeriosis is the more severe form of the disease and affects particularly certain high-risk groups of the population. These include pregnant women and their newborns, the elderly, and people with weakened immune systems (EURL Lm, 2021). *L. monocytogenes* infections are most commonly reported in the age group 'over 64 years' and particularly in the age group 'over 84 years'. Spain is the third country in the EU with the highest number of confirmed cases of listeriosis with 362 cases (EFSA and ECDC, 2021).

Among the category of meat products, higher risk for public health is associated with meat consumed as ready-to-eat (RTE) or without a proper cooking. Global RTE meat products market is expected to grow at a CAGR of 4.8% during the forecast period 2019–2026 (https://straitsresearch.com/report/ready-to-eat-meat-products-market/). RTE meat products comprise manufactured, processed and fermented meat for direct consumption and include products such as beef jerky, pepperoni, salami, smoked deli meats and cured ham. *L. monocytogenes* occurs frequently in fresh pork meat at low or moderate levels and the pathogen may become established in the processing environment and cross-contaminate meat products (Thévenot et al., 2006). The ability of this microorganism to grow at refrigerated temperatures (≥ -0.4°C), at a pH between 4 and 9.6 (Välimaa et al., 2015), and water activities ≥ 0.92 in additions to its moderately high tolerance to salt, makes it very dangerous in a wide variety of RTE meat products (Burnett et al., 2005; Kurpas et al., 2018). Considering that the probability of infection is correlated to the quantity of serving, Spain is considered a country at risk since the consumption of RTE meat products is one of the highest in the EU with almost 1 kg/month per person (Celada et al., 2016).

The RTE meat production chain is complex and there are different possible sources of *L. monocytogenes* contamination in each step. Reduction or elimination of the microorganisms during processing will be helpful in limiting the bacteria in the food at the time of consumption. The risk associated with RTE products depends mainly on the effectiveness of control measures implemented by food business operators (FBOps), including Good Agricultural Practices (GAPs) at primary production, Good Manufacturing Practices (GMPs) and HACCP programmes at processing and retail. However, the ability of this microorganism to survive under extremely different environmental conditions and the fact that *L. monocytogenes* can form biofilms (Lee et al., 2019) on the surfaces of food industries make its control very challenging. Microbiological criteria for RTE foods is defined by Regulation (EC) No 2073/2005 and it indicates for products supporting the growth of *L. monocytogenes* to satisfy that the product will not exceed the limit 100 CFU/g throughout the shelf-life. The infectious dose in invasive listeriosis is not well known (Swaminathan and Gerner-Smidt, 2007).

1.2. Conventional and innovative food processing for the inactivation of *L. monocytogenes* in RTE meat

Food processing technologies can be applied to reduce the probability of *L. monocytogenes* being present in RTE meat. Common thermal treatments, such as pasteurisation and sterilisation, are effective but can be applied only in cooked meat, while raw-derived products, such as cured ham would be affected by undesirable changes in sensorial characteristics. On the contrary, irradiation might be applied for sterilisation with good results on the sensorial acceptance (Jin et al., 2012), but its use in the EU is limited only to a few product categories by Directive 1999/2/EC and 1999/3/EC. Moreover irradiation could pose concerns to consumers (Cardello et al., 2007). The use of food preservatives and/or antimicrobial additives are common approaches to control *L. monocytogenes* growth in RTE meat and extend shelf life. However, they are almost ineffective to kill *L. monocytogenes* and their use can not exceed some precaution level by law (Dussault et al., 2016). For all these reasons, new processes are highly desirable.

In the last decades, alternative mild and non-thermal processing technologies have been developed to increase food safety without altering the product's quality. Within these technologies, high hydrostatic processing (HHP) is shown to be effective against *L. monocytogenes* inoculated in cured ham (Morales et al., 2006) without altering the sensory characteristics. The process needs very high pressure (> 300 MPa) which might discourage its applications in small enterprises and retails due to the high cost of machinery. Alternatively, the use of carbon dioxide at supercritical state $ScCO_2$ has also been successfully applied to inactivate *L. monocytogenes* on the surface and on slices of cured ham (Ferrentino et al., 2013). $ScCO_2$ has a low critical point (304.1 K, 7.38 MPa) which allows handling processes at relatively low-pressure conditions in comparison to HHP, thus lower investment costs. CO_2 can penetrate inside the tissue and inactivate microorganisms at the surface and internally. CO_2 has been defined as GRAS (Generally Regarded As Safe) by FDA, it is obtained by waste production and is fully recycled. CO_2 is a relatively inert gas, not affecting the other food compounds, non-toxic, nonflammable and easily removable after the process without any contaminants or residues. Consequently, it is approved for food processing without declaration (Brunner, 2005). However, the adoption of novel technologies in the food industry requires strong scientific evidence to drive its acceptance at industrial level. In this context, risk assessment can be used to support the development and use of innovative processing and identify any scientific/regulatory gap, which might be needed for its commercialisation.

1.3. Quantitative microbial risk assessment

Quantitative microbial risk assessment (QMRA) is a science-based process, which estimates the risk of infection and illness when a population is exposed to microorganisms in food (Mataragas et al., 2010). QMRA follows four steps: hazard identification, hazard characterisation, exposure assessment and risk characterisation. In hazard identification, scientists identify biological hazards (such as bacteria, viruses, parasites, fungi and moulds or their toxins) that could be present in food. Hazard characterisation studies the nature of the health effects of the hazard (e.g. pain) and, where possible, proposes a dose–response model. In the exposure assessment, there is an association of the hazard present in the food with the population and the amount of food eaten. In the risk characterisation, assessors draw conclusions on the likelihood of the health effect of the specific pathogens for consumers in general or specific groups. For QMRA implementation, it is necessary to develop mathematical models for the analysis of the behaviour of the risk along the whole food chain. The likelihood of the event is usually described using stochastic methods, which include uncertainty and variability in the analysis (Thompson, 2002). In the last few years, different methodologies have been proposed to conduct QMRA and models and software are available for these studies.

2. Description of work programme

2.1. Aims

The main objective of EU-FORA is to enhance cooperation among Europe's food safety organisations and between them and EFSA, thus contributing to the harmonisation of food risk assessment practices across Europe. Specifically, this EU-FORA project was focused on the development of training and research activities to perform QMRA and evaluate the risk associated with the consumption of RTE foods. Considering the background of the fellow in innovative food processing, the training included the integration between the existing knowledge of the hosting institution and the fellow within the QMRA field, aiming at fostering the transfer of knowledge on a well know based environment and establishing a long-term study in this field that can be continued at the end of the programme. A synergistic research activity was carried out between the Polytechnic University of

Cartagena (UPCT) in Spain and the University of Padua (UNIPD) in Italy to promote the transfer of knowledge during and after the programme.

2.2. Activities/Methods

The activity focused on training in QMRA methodologies to cover the four steps of risk assessment (hazard identification, hazard characterisation, exposure assessment and risk characterisation). As part of the training, a practical case study based on a common RTE Spanish product (cured ham) has been used for the implementation, and different scenarios were identified and discussed. Among them, the use of innovative food processing technologies as an additional decontamination step, specifically the use of $ScCO_2$ pasteurisation, was considered to increase the product's food safety. As a hazard, *L. monocytogenes* was considered the most relevant pathogen in RTE meat. The description of the main activities carried out during the programme is reported below.

2.2.1. Training on methodologies related to risk assessment

These activities have provided the theoretical background and basic skills to perform a QMRA, complementing the education and knowledge acquired during the training modules by EFSA. Thanks to the incorporation in a working team with proven expertise in the use of risk assessment tools, the fellow received training on specific topics such as:

i) Handling of available databases (EFSA, FAO, the group's database for microbial inactivation);
ii) Optimal experimental design (including the bioOED software developed in the group) applied to growth and inactivation experiments;
iii) Growth and inactivation modelling (Combase and Bioinactivation developed in the group);
iv) Statistical analysis using Monte Carlo and Bayesian methods and risk ranking methodologies;
v) Software tools specific for risk assessment (MicroHibro, FDA-iRISK, biorisk);
vi) Training on the use of the R programming language and Rstudio.

2.2.2. Collecting data from literature and laboratory work

This activity provided knowledge on methodologies for data collection to characterise the microbial response along the food chain. Data collection is a key fundamental phase in QMRA because it allows the assessors to understand the current state of the art and, if necessary, to drive specific experimental campaigns when key data are missing. The collection included both literature search from available databases, scientific papers, reports and laboratory activities. The data needed for QMRA include inactivation kinetics of the relevant microorganism in industrial processing, as well as the growth during storage conditions (e.g. transport, retails, domestic). The use of inactivation processing technologies was also included in the study considering both traditional heat treatments and innovative non-thermal or low-temperature pasteurisation technologies. During this task, the fellow was trained on the use of a thermoresistometer available at UPCT applying Optimal Experiment Design methodologies already developed in the group. A Mastia thermoresistometer was used to study the inactivation kinetic behaviour in isothermal or dynamic experimental conditions of a microbiological strain (Conesa et al., 2009). For the enumeration, standard plate count technique was used using selective or non selective media using a colony counter. The fellow was also trained on the use of the HHP machine available at UPCT, performing exploratory experiments with packaged products in modified atmosphere packaging (MAP).

2.2.3. Development of mathematical models

Mathematical modelling is a common tool in food science and technology. The microbial behaviour along the food chain (prevalence, growth, inactivation, acclimation, etc.) was described using both existing models reported in literature and *de novo* models implemented based on data gathered from literature and/or novel experimental data. The prevalence levels of *L. monocytogenes* in dry cured meat is highly dependent on the type of meat. In case of cured ham the overall occurrence of *L. monocytogenes* in retail varies from not detected to a prevalence up to 12% (Serra-Castelló et al., 2020). In Spain, the prevalence in dry-cured ham deboning and slicing areas was 9.16% (Alía et al., 2020). The growth of *L. monocytogenes* can be modelled depending on storage temperature and water activity though secondary polynomial models (Serra-Castelló et al., 2020). Inactivation by innovative processing was analysed using Bioinactivation software (Garre et al., 2017) which allows to predict the inactivation process of a population of microorganisms after an inactivation process. Inactivation of *L. monocytogenes* in cured ham with $ScCO_2$ (Ferrentino et al., 2013) was fitted using the Peleg model (Peleg and Cole, 1998) and can achieve up to 6 logCFU/g inactivation. Reductions on *L. monocytogenes* in low water activity dry-cured ham after HHP process at 600 MPa for 5 min was found moderate (Pérez-Baltar et al., 2021), and not considered in further studies.

2.2.4. Estimation of the risk based on different scenarios

The exposure assessment model for risk characterisation was elaborated according to the methodology by Codex *alimentarius* for QMRA. An exponential dose–response model by FAO/WHO was selected. Exposure assessment was implemented using the statistical software R applying a new package 'biorisk' developed by the group at UPCT and available in GitHub (https://github.com/albgarre/biorisk/). Other existing web-based tools (MicroHibro, FDA iRisk) were also used during the training. The data gathered from literature, together with the kinetic models was implemented using probability distributions including the relevance of variability and uncertainty, according to the recommendations of international food safety agencies. The risk was calculated on the basis of a stochastic model and solved using Monte Carlo simulations. The exposure assessment provides an estimate of the occurrence and level of the pathogen in a specified portion of food at the time of consumption, aiming to calculate the probability of consuming a given dose of the pathogen of interest. The exposure assessment considered the most relevant stages of the food chain, and included processes and storage until the consumption. Different scenarios can be defined by changing the process stages or the initial concentration or the dose of serving, permitting to consider the adoption of better practices which can reduce the risk for the population.

2.3. Secondary activities

Apart from the mentioned tasks, additional training and other activities were carried out/provided in person and/or remotely. This helped the further improvement of the skills on risk assessment and permitted to carry out communication, dissemination and exploitations activities. Moreover, the transfer of knowledge on topics related to food safety has been provided during the whole time thanks to the involvement in co-supervision and mentoring of PhD students, undergraduate and graduate students in topics related to risk assessment, food processing and data analysis. The submission of national and international research grant proposals was also carried out on topics related to innovative food processing and risk assessment with the involvement of the supervisor in the research team. Following are some details of the most important activities presented.

2.3.1. Visit to AESAN

A few days visit in the headquarters of the Spanish Agency for Food Safety and Nutrition (AESAN) on 23–24 February 2022, in Madrid and on 25 at the 'Centro Nacional de Alimentación' (CNA) in Majadahonda was organised for the fellows placed in Spain. During this visit, there was the opportunity to present the EUFORA activities to the AESAN Scientific Committee and meet in person all the other fellows. Moreover, seminars were organised for the fellows to present the AESAN's work on risk assessment, management (biological risk, chemical risks, nutritional safety) and communication. It was also explained the official food control and alerts and the Spanish strategy for nutrition, physical activity and the prevention of obesity (NAOS). During the visit at the laboratory at CNA, the activities carried out on food contact materials, toxin and food processing contaminants, residues of veterinary drugs, biotechnology and microbiology and antimicrobial resistance were explained with practical examples.

2.3.2. Participation in conferences/webinars/meetings

1) Webinar - Horizon Europe Cluster 6 'Food, Bioeconomy, Natural Resources, Agriculture and Environment' Information Days organised by the European Research Executive Agency, 25–26 October 2021, online.
2) Webinar 'Beginner's Guide to Using Sequencing to Facilitate Microbial Research' organised by Novogene, 11 November 2021, online.
3) NETVAL Webinar Training Orbit Intelligence, 1 December 2021, online.
4) RIBMINS 2nd Scientific Conference 'Towards the future of meat safety assurance', 7–8 April (2022) hybrid conference in Córdoba (Spain), online participation.

5) VIII National Congress of Industrial Microbiology and Microbial Biotechnology, 1–4 June 2022, Valencia (Spain), hybrid event, participation in person with the presentation of the poster 'Tools for quantitative microbial risk assessment along the food chain' (Annex A).
6) ONE – Health, Environment, Society – Conference 2022, 21–24 June 2022 in Brussels and online (online participation).
7) Symposium on Aerogels for Food Applications within the 2nd International Conference on Aerogels for Biomedical and Environmental Applications, 29 June–1 July 2022, Athens, participation in person.

2.3.3. Lecture and seminars to students

A seminar entitled 'Supercritical CO_2 technologies for the pasteurization and drying of food' has been organised the 27 October 2021 at UPCT for the TAIDA DOCTORAL PROGRAMME (face to face and online) addressed to PhD and master's students and researchers. The research activity on QMRA and risk assessment has been presented to the students of the courses 'Separation unit operations and process simulation' (online, 11 January 2022) and 'Food and Bioprocess Technology' (face to face and online, 24 May 2022) enrolled to the master degree in Chemical Engineering and Industrial Processes at UNIPD. A lecture entitled '$HPCO_2$ pasteurization and drying of food product' has been presented to international PhD and master students the 9th July in Maribor (Slovenia) during the Green Engineering by High Pressure Technology (GEHTP) summer school (COST action GREENERING).

3. Conclusions

3.1. Scientific outcomes

The development of tools to allow the easy implementation of exposure assessment along the food chain is important to harmonise practices and support risk assessors and decision-makers on the recommendation of the future commercial food production. The risk of listeriosis due to the consumption of RTE meat could be slowed down by implementing better practices of handling and storage to control the cross-contamination and growth of listeria. Another solution might be the development of new gold standard processes to reduce the risk of cross-contamination by inactivating the pathogens before slicing without altering the product quality. In this regard, the use of $ScCO_2$ pasteurisation technologies could be a suitable innovative technology for RTE meat. Data on the use of low-temperature pasteurisation technologies showed a reduced risk at the time of consumption. However, the implementation of such technologies should also consider economic aspects and the return of investment. Their adoption might require financial support by EC to SMEs and retailers to promote their use as a standard decontamination process.

Even if the use of low-temperature pasteurisation might be useful to increase food safety, more and more scientific evidence is needed to support their development. Specifically in case of $ScCO_2$ pasteurisation, after reviewing the current state of the art, it has been highlighted that little is known about the inactivation mechanism on solid food, and information about the behaviour of pathogenic microorganisms as well as inactivation data which can be modelled and used for QMRA. A comprehensive review on the current state of the art of the technology for the increase of the safety of meat is underwriting.

3.2. Participation of the programme

The overall focus of the work programme was the training on food risk assessment, thanks to the collaboration with a competent research group in food safety. During the programme, specific training on topics related to food safety and risk assessment has been provided by EFSA and partners within the participation of five modules of lectures and workshops. The fellow became familiar with methodologies, terminologies and practices commonly used in food risk assessment. Thanks to the EU-FORA, the fellow had the opportunity to learn by practice and apply the concept learnt to the field of innovative food processing for pasteurisation and drying, such as inactivation models and tools for the prediction of microbial behaviour. The participation in the EU-FORA programme allowed to expand the scientific network and start building cooperation and collaboration across Europe, which will be fundamental to establish interdisciplinary research focused on the future food processing industry. After the participation of the programme, the fellow will transfer the new knowledge to his research group in Italy by training students and new fellows in the field of food safety and risk assessment. The programme was an incredible opportunity for personal and professional growth having a strong impact in the future career.

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Abbreviations

AESAN	Spanish Agency for Food Safety and Nutrition
CAGR	compound annual growth rate
CFU	colony forming unit
CNA	Centro Nacional de Alimentación
CRAN	Comprehensive R Archive Network
EU-FORA	European Food Risk Assessment
FBOp	food business operator
FDA	Food and Drug Administration
GAP	Good Agricultural Practices
GMP	Good Manufacturing Practices
GRAS	Generally Regarded As Safe
HACCP	Hazard Analysis and Critical Control Points
HHP	high hydrostatic pressure
$HPCO_2$	high-pressure carbon dioxide
MAP	modified atmosphere packaging
NAOS	Spanish strategy for nutrition, physical activity and prevention of obesity
NETVAL	network for the enhancement of research
PhD	Doctor of Philosophy
QMRA	quantitative microbial risk assessment
RIBMINS	risk-based meat inspection and integrated meat safety assurance
RTE	ready-to-eat
$ScCO_2$	supercritical carbon dioxide
STEC	Shigatoxin-producing *Escherichia coli*
TAIDA	Técnicas Avanzadas en Investigación y Desarrollo Agrario y Alimentario
UNIPD	University of Padua
UPCT	University Polytechnic of Cartagena
WHO	World Health Organization

Annex A – Poster presented at the VIII National Congress of Industrial Microbiology and Microbial Biotechnology

TOOLS FOR QUANTITATIVE MICROBIAL RISK ASSESSMENT ALONG THE FOOD CHAIN

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ABSTRACT

The aim of this work is to present an example of implementing tools for Quantitative Microbial Risk Assessment (QMRA) of food products along the food chain. A common ready-to-eat (RTE) product from Spain was used as a case study to identify a possible microbiological hazard for the population. Following, the exposure assessment model was implemented using mathematical models and statistical software to describe the microbial behavior along the food chain. The study will present the possibility to identify the risk exposure in different scenarios (e.g. growth during different storage conditions, inactivation induced by decontamination techniques), showing the flexibility of the tools. The outcome of the QMRA is to estimate the probability of illness of the target population after the consumption of possible contaminated food.

1. INTRODUCTION

Quantitative Microbial Risk Assessment (QMRA) is a scientific based process, which estimate the risk of infection and illness when a population is exposed to microorganisms in food. QMRA follows four steps (Fig 1). For QMRA implementation, it is necessary to develop mathematical models for the analysis of the behavior of the risk along the whole food chain. Here an example of implementing QMRA is reported considering as case study the risk for *Listeria monocytogenes* in RTE cured meat in combination with the adoption of low temperature pasteurization technologies.

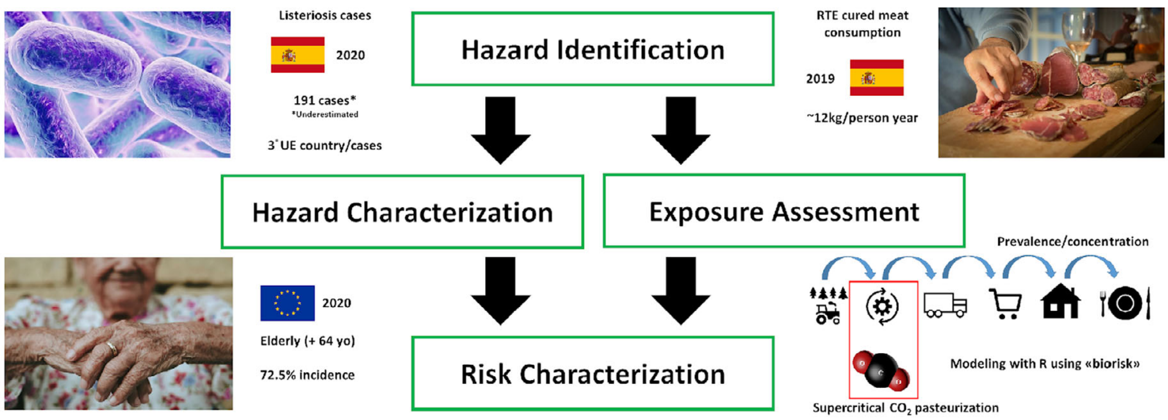

Figure 1. The four steps of risk assessment. Hazard characterization studies the nature of the health effects of the hazard (e.g. pain) and where possible propose a safe level of exposure for consumers. In the exposure assessment, there is an association of the hazard present in the food with the population and the amount of food eaten. In the risk characterization, assessors draw conclusions on the level of risk of the specific pathogens for consumers in general or specific groups

2. METHODS

The risk assessment was based on four separate stages in accordance with Codex Alimentarius. Hazard identification was performed collecting information from literature data on listeriosis and the behavior of *L. monocytogenes* in cured meat. For the hazard characterization the exponential dose-response model by FAO/WHO was selected. Growth and inactivation modelling were estimated using Combase and Bioinactivation [1]. Exposure assessment was implementing using the statistical software R and the package biorisk. The risk was characterized per number of serving.

3. RESULTS

The prevalence levels of *L. monocytogenes* in dry cured meat is highly dependent on the type of meat and post process operation, which is responsible of cross contamination. In case of dry cured ham the overall occurrence of *L. monocytogenes* in retail varies from not detected to a prevalence up to 12% [2]. *L. monocytogenes* can growth at low temperature during storage. The inactivation kinetic of *L. monocytogenes* by high pressure CO_2 in spiked dry ham at 8 and 12 MPa [3] was modeled using Weibull-Peleg inactivation model. Different pathways can be implemented for the exposure assessment, including growth at different stages (e.g transport, grocery, domestic) and including decontamination phases. Including data on consumption and threshold vales for specific group of people, it is possible to estimate the level of risk. The model permits to evaluate different case scenario in which the adoption of best practices can reduce the risk for the population.

4. CONCLUSIONS

L. monocytogenes is a harmful pathogens. In 2020, 27 MS reported 1,876 confirmed invasive human cases of *L. monocytogenes* that caused 780 hospitalizations and 167 deaths in the EU. Listeriosis was the fifth most commonly reported zoonosis in humans in the EU. *L. monocytogenes* infections were most commonly reported in the age group over 64 years' and particularly in the age group over 84 years'. The development of tools to easily implement exposure assessment along the food chain is important to harmonize practices and support risk assessors and decision-makers on the recommendation of the future commercial food production.

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6. REFERENCES

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