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Listeria monocytogenes contamination of ready-to-eat foods and the risk for human health in the EU

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Abstract

Around 2,200 confirmed human listeriosis cases were reported in the European Union and European Economic Area (EU/EEA) in 2015. Time series analysis for the EU/EEA indicated an increasing trend of the monthly notified incidence rate of confirmed human listeriosis in female age groups over 25 years old, and in the male ≥ 75 age group during 2008–2015. In 2015, the incidence rates were higher for the male than for female age groups over 45 years old and the opposite for the 15-24 and 25–44 age groups (mainly pregnancy-related). Incidence rates were highest for the ≥ 75 age group. A conceptual model was the basis for identifying factors in the food chain as potential drivers for *Listeria monocytogenes* contamination of ready-to-eat (RTE) foods and listeriosis illness. The factors related to the host (i. population size of the elderly and/or susceptible people; ii. underlying condition rate), the food (iii. *L. monocytogenes* prevalence in RTE food at retail; iv. *L. monocytogenes* concentration in RTE food at retail; v. storage conditions after retail; vi. consumption), the national surveillance systems (vii. improved surveillance), or the bacterium (viii. virulence). Their potential contribution to the trend of human listeriosis cases/incidence rates were evaluated by answering eight assessment questions through importance analysis using a developed *L. monocytogenes* generic quantitative microbiological risk assessment (gQMRA) model. Factors that may have had an impact on the trend were classified based on the quality of available evidence. Among the evaluated factors, those considered likely to be responsible for the increasing trend in cases are the increased population size of the elderly and susceptible population except for the 25–44 female age group. For the increased incidence rates and cases, the likely factor is the increased proportion of susceptible persons in the age groups over 45 years old of both genders.

Keywords: ready-to-eat food products, *Listeria monocytogenes*, human listeriosis, time series analysis, quantitative microbiological risk assessment

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Summary

In 2010–2011 an EU-wide baseline survey (BLS) estimated the prevalence and concentration of *Listeria monocytogenes* in three ready-to-eat (RTE) foods at retail: packaged (not frozen) smoked or gravad fish, packaged heat-treated meat products and soft or semi-soft cheese. The EU-level estimate of the proportion of samples with *L. monocytogenes* counts exceeding the level of 100 colony forming units (CFU) per gram at the end of shelf life was 1.7% for 'RTE fish,' 0.43% for 'RTE meat' and 0.06% for 'RTE cheese.' Despite the application of the new food safety criteria (FSC) for *L. monocytogenes* in (RTE) foods from 2006 onwards (Commission Regulation (EC) 2073/2005[1]) and the outcome of the BLS, a statistically significant increasing trend of human listeriosis was reported in the European Union and European Economic Area (EU/EEA) over the period 2009–2013 (EFSA and ECDC, 2015).

Therefore, the Panel on Biological Hazards of the European Food Safety Authority (EFSA) initiated a self-tasking mandate to deliver a Scientific Opinion on *L. monocytogenes* contamination of RTE foods and the risk for human health in the EU. The Opinion draws conclusions on the two terms of reference (ToR): (1) to summarise and critically evaluate the most recent information on *L. monocytogenes* in RTE foods and (2) to discuss and evaluate the factors related to contamination in the food chain and the consumption patterns that may contribute to the reported trend of listeriosis incidence rates in the EU. The focus was on the time period after the adoption of the previous Scientific Opinion of the BIOHAZ Panel at the end of 2007, i.e. 2008–2015 (EFSA BIOHAZ Panel, 2008).

For the **ToR 1** in particular, the following sources were to be considered: (a) the above-mentioned BLS and the monitoring data and (b) the three EFSA outsourcing activities under 'Closing gaps for performing a risk assessment on *L. monocytogenes* in RTE foods,' i.e. (i) the presence of, and risk factors for, *L. monocytogenes* in RTE foods in the EU, (ii) the estimation of the public health risks from consumption of various RTE food categories contaminated with *L. monocytogenes*, and (iii) the comparison of *L. monocytogenes* isolates from different compartments along the food chain, and in humans using whole genome sequencing (WGS).

It is highlighted that, despite an increase in confirmed listeriosis cases during 2008–2015, fewer than 2,300 cases per year were reported in the EU/EEA. The notified incidence rates of invasive listeriosis in the EU/EEA generally increased with increasing age, and were highest in the age groups over 65 years and in children below 1 year of age (i.e. mainly pregnancy-related cases). In addition to age/susceptibility, medical practices for other ailments have been hypothesised as risk factors for human listeriosis, such as treatments with proton pump inhibitors (PPI). Bloodstream infections were the most commonly reported clinical forms of invasive *L. monocytogenes* infections (71.8% of confirmed cases), followed by meningitis (19.4% of cerebrospinal fluid samples), and the overall annual case fatality rates (CFR) ranged from 12.7 to 20.5%.

There is ample evidence for a high variability regarding the virulence potential and pathogenicity of *L. monocytogenes* isolates. Epidemiological data combined with genetic sequencing information and results from animal models (> 6,000 isolates from clinical specimens and food items) indicate that only 12 clonal complexes (CC) make up almost 80% of all isolates, and that different levels of virulence may be associated with these. Listeriosis is a food-borne illness, but CCs have been termed, according to one study, 'infection-associated,' 'food-associated' or 'intermediate' depending on the relative proportion of isolates from clinical cases, food or both. Uncertainty may be associated with this classification due to knowledge gaps about factors influencing the isolation and detectability of different strains from different matrices. 'Infection-associated' CCs are most commonly associated with central nervous system (CNS) and maternal–neonatal (MN) infections as opposed to bacteraemia alone, while 'food-associated' CCs are rarely isolated from clinical samples but, when recovered from clinical specimens, usually isolated from blood. In addition, 'food-associated' CCs are more frequently associated with highly immunocompromised patients or patients showing a higher number of severe comorbidities. Based on humanised mouse models it appears that these predominately 'food-associated' CCs are less invasive (hypovirulent) than the 'infection-associated' CCs. However, despite

[1] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, p. 1–26.

the observed variability in their virulence potential, any *L. monocytogenes* strain has the ability to result in severe human listeriosis because of the complex interaction between the pathogen, food and host.

The fact that most listeriosis cases appear to be sporadic, and reported outbreaks are commonly small, making it difficult to detect links between human cases and causative foods. However, it has been suggested that next generation sequencing (NGS) techniques may have the potential to improve this detection. Results from the outsourced study to attribute human cases to different animal sources are limited not only by the representativeness of isolates from all relevant sources but also by difficulties of identifying their origin, since contamination during processing is so important. Persistence of *L. monocytogenes* in food processing environments is considered to be the major source of RTE food contamination. Persistence appears to be the result both of improper hygiene conditions and the high adaptive capacity of these bacteria against physical–chemical factors, for example, biofilm-forming capacity.

The RTE food categories typically associated with human listeriosis, i.e. 'meat and meat products,' 'fish and fish products,' and 'milk and milk products' continue to have a significant public health impact. In addition, food of plant-derived origin or even frozen foods have been implicated in outbreaks (e.g. cantaloupe, caramel apples, ice cream) illustrating that almost all RTE foods under certain unexpected conditions may support growth and/or that when consumed by highly susceptible people, have the potential to contribute to the burden of disease. During the period 2008–2015, reported annual non-compliance of *L. monocytogenes* in RTE foods at processing sites was highest in 'RTE fishery products' (3–10%), followed by 'RTE products of meat origin other than fermented sausage' (1–7%). Non-compliance in the remaining RTE food subcategories was 2% or less. The lower level of annual non-compliance at retail (below 1% for most years) than at processing is at least partly explained by the application of the different limits of FSC at retail and processing.

According to the BLS, as presented above, *L. monocytogenes* was more prevalent in 'RTE fish': 10.3% (1.7% above 100 CFU/g) than in 'RTE meat': 2.1% (0.43% above 100 CFU/g) and 'RTE cheese': 0.5% (0.1% above 100 CFU/g) at the end of their shelf life. Cooked meat and heat-treated sausages were the RTE food subcategories with most consumed servings per person and per year in the EU/EEA. Combining the BLS data with consumption data indicates that approximately 55 million servings of RTE meat and meat products contaminated with more than 100 CFU/g may be consumed per year by the population over 75 years old in the EU/EEA.

It was noted that unsafe practices (including storage time and temperatures) are not uncommon within the elderly group (> 10% of persons studied), and have a potential impact on the human listeriosis risk. There is a wide variation within the broadly defined consumer groups and it is thus problematic to generalise about the food handling behaviours of these groups and on how this may contribute to trends of human listeriosis. In addition, the temperature of domestic refrigerators is highly variable as shown through a review of 23 available survey studies from 1991 to 2016. The mean, minimum and maximum temperatures ranged from < 5 to 8.1°C, -7.9 to 3.8°C and 11.4 to 20.7°C, respectively. The extent of different behaviours among risk groups between Member States may vary to the same extent that socioeconomic factors, traditions and types of food vary. Since the majority of studies of food handling are from a few countries only, this may lead to some uncertainty.

The average probability of a single *L. monocytogenes* CFU to cause illness in a specific host (the *r* value), reflects the strain virulence and host susceptibility, and ranges three orders of magnitude, from the least to the most susceptible subpopulations. Reported *r* values for specific outbreaks with highly susceptible populations increase the range by another five orders of magnitude. This means that the probability of a single CFU to cause illness may range 100 million times depending on variability in host susceptibility and *L. monocytogenes* virulence. A lognormal-Poisson extension of the exponential dose–response (DR) model, incorporating the virulence and susceptibility variability for 11 population groups, suggests that most listeriosis cases are linked to the ingestion of food contaminated with medium to high (3.5 – 7.5 $\log_{10}$ CFU/serving) *L. monocytogenes* concentrations.

Most risk characterisations considered three risk populations (i.e. pregnant women/perinatals, the elderly (> 60 or > 65 years old), and the intermediate population that does not belong to either of these categories) and have not addressed gender differences. This limitation can be addressed with DR data and other input data developed at a finer resolution in recent publications and in the present Opinion.

Based on the quantitative risk characterisation of *L. monocytogenes* in various RTE food categories (heat-treated meat; smoked and gravad fish; and soft and semi-soft cheese) in the EU, it was concluded that most of the cases were predicted in the elderly population (≥ 65 years old) (48% of cases) followed by the pregnant population (41%) and the healthy population < 65 years old (11%). The food subcategory associated with the largest number of cases per year was cooked meat (863 cases), followed by sausage (541 cases), gravad fish (370 cases), cold-smoked fish (358 cases), pâté (158 cases), soft and semi-soft cheese (19 cases) and hot-smoked fish (7 cases). Estimated risks expressed as the median number of cases per 10^6 servings was in general highest for the pregnant population, followed by the elderly and last the healthy (< 65 years) population. Uncertainty sources for some variables such as initial prevalence of *L. monocytogenes* in RTE foods should be further elucidated as well as variability in *L. monocytogenes* growth when types of product and populations are compared.

To address **ToR 2**, for the time period 2008–2015, time series analyses (TSA) of 14,002 confirmed human listeriosis cases in the EU/EEA were carried out at different levels of aggregation, i.e. aggregated by total confirmed cases, and disaggregated by 14 age–gender groups. The aggregated TSA did not show an increasing trend, which is partly a consequence of the presence of changing dynamics, autocorrelation and strong seasonality in the aggregated analysis. This is in contrast to the disaggregated analyses for which clear trends were shown and where some of the aforementioned characteristics were present to a lesser extent.

For females, the incidence rate of confirmed human listeriosis significantly increased for the 25-44 and ≥ 75 age groups in this time period with a monthly increase estimated at 0.64% and 0.70%, respectively. For the female age groups 45-64 and 65-74 the increasing trend was borderline significant with a monthly increase estimated at 0.43% and 0.30%, respectively. For males, the incidence rate of confirmed human listeriosis cases increased significantly for the ≥ 75 age group only with a monthly increase estimated at 0.50%. In 2015, the listeriosis incidence rate was higher for males than for females in the age groups over 45 years old. The opposite was true for the female age groups 15-24 and 25–44 believed to largely reflect pregnancy-related listeriosis. The highest incidence rate was seen in the ≥ 75 age group in 2015, resulting in an incidence rate of 2.20 and 1.30 cases per month per million persons for males and females respectively. There are several sources of uncertainty, which can lead to under- or overestimation of the observed trends. Because of the limitations of the available data, the analysis and understanding of trends were carried out using age and gender as proxies for susceptible populations and did not include countries as a covariate. This is a limitation and means that the observed trends may hide different trends among subgroups or be true for only a subset of the age–gender–country population.

Potential factors related to the human host, the food, the national surveillance systems, or the bacterium, to be addressed in ToR 2 to explain the epidemiological trend were identified via a conceptual model. The selected factors were evaluated as assessment questions (AQs) in three steps. First, an importance analysis was used to evaluate the most important factors and their potential impact on the number of predicted cases using a developed *L. monocytogenes* generic quantitative microbiological risk assessment (gQMRA) model. Second, the empirical evidence, i.e. the indicator data, was evaluated to investigate the support for a change in the factor during the time period. Third, an evidence synthesis of the TSA, the importance analysis, indicator data and the uncertainty analyses was made.

It was indicated that 92% of listeriosis cases for all subpopulations are attributable to doses above 10^5 CFU per serving. Assuming an average serving size of 50 g, this would correspond to an average *L. monocytogenes* concentration in RTE foods of 2,000 CFU/g at the time of consumption. Based on predictions of the gQMRA model, the expected number of listeriosis cases per year is reduced by 37% (from 1,523 to 953) in the absence of growth from retail onwards. The frequency of exposure (i.e. the prevalence of *L. monocytogenes* in RTE food) over 25 years old appears to increase with increasing age for both genders, due to differences in consumption patterns.

Factors that may have contributed to the increasing trend of human listeriosis cases/incidence rates in the EU/EEA during 2008–2015 were classified, based on the potential impact when changing the factor according to modelling or other information, the degree of support from indicator data, and expert opinion, into probability scales as defined in the draft EFSA guidance on uncertainty (EFSA Scientific Committee, 2016).

- The first **likely** (66–90%) factor was an increased proportion of susceptible persons in age groups over 45 years for both genders. The increasing trend in the female 25–44 age group (mainly pregnancy-related) suggests that a factor other than susceptibility must have contributed since susceptibility is not expected to have changed in this population during the time period. The additional factor may be any of those evaluated and would likely contribute to the trend in all age groups but possibly to a varying degree. The second likely factor is an increased population size of the elderly and susceptible population (except in the female 25–44 age group which has decreased). This factor would only contribute to the number of listeriosis cases but not the increase in incidence rates.
- The factors considered as **likely as not** (33–66%) were an increased consumption (number of servings per person) of RTE foods in the EU/EEA and an improved surveillance of human listeriosis in the EU/EEA.
- **Inconclusive** factors were: (1) *L. monocytogenes* **concentration** in the three considered RTE food categories at retail; (2) *L. monocytogenes* **prevalence** in the three considered RTE food categories at retail; (3) *L. monocytogenes* **virulence** potential; (4) **storage conditions** (time and temperature) after retail of the three considered RTE food categories.

Due to data limitations the present evaluation was based on only three RTE categories which is a limitation of the assessment. Uncertainty is associated with the gQMRA model because of data and knowledge gaps. An important source of uncertainty is the dose–response relationship since it is dependent on the same data as used in the exposure assessment and the epidemiological data. However, the impact of uncertainty is expected to be lower for the importance analysis when the relative effects of factors were evaluated than for the absolute number predictions.

Data gaps include representative data collected across the EU/EEA using a harmonised sampling strategy suitable for surveillance over time on: (1) prevalence and concentration of *L. monocytogenes* in RTE foods; (2) consumption of RTE foods; (3) prevalence of different risk groups by age and gender; (4) retail and home storage temperatures; and (5) *L. monocytogenes* virulence.

It was recommended that awareness be raised among all stakeholders in the food chain about the potentially increasing problem of *L. monocytogenes* in RTE foods since the proportion of European citizens in high-risk groups is expected to increase in the EU/EEA. The implementation of innovative programmes to generate data on *L. monocytogenes* in food that are comparable across Member States and time in the EU was also recommended; existing monitoring has other objectives and is not appropriate for evaluating trends over time. A further recommendation is to address the need for data to evaluate changes over time in the consumption of RTE foods and other food categories in the EU. Also, improvement of the information for risk assessment and risk management was recommended. This can be achieved by collecting comparable data on human listeriosis cases that are more aligned with the concepts of risk groups in terms of the number of cases within these groups and their consumption habits as well as socioeconomic–demographic data. Finally, it was recommended that better information should be obtained on how the dietary practices and food handling among elderly groups are affected by ageing and how this may be linked to an increased exposure to *L. monocytogenes*.

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1. Introduction

1.1. Background and Terms of Reference (ToR) as provided by the requestor

On 1 January 2006, Commission Regulation (EC) 2073/2005[2] became effective for all European Union (EU) Member States defining, among others, new food safety criteria (FSC) for *Listeria monocytogenes* in ready-to-eat (RTE) foods.

An EU-wide baseline survey (BLS) was conducted in 2010 and 2011 to estimate the prevalence and contamination levels of *L. monocytogenes* in three RTE foods at retail in accordance with Decision 2010/678/EU[3]: packaged (not frozen) smoked or gravad fish (3,053 samples), packaged heat-treated meat products (3,530 samples) and soft or semi-soft cheese (3,452 samples). This survey showed levels of compliance with the FSC for the selected groups of RTE foods as follows: 98.3%, 99.5% and 99.9% for fish, meat and cheese samples, respectively (EFSA, 2013, 2014).

Despite the application of the new FSC for *L. monocytogenes* from 2006 onwards and the level of compliance for certain RTE foods in the BLS, 27 Member States reported 1,763 confirmed human cases of listeriosis and 191 deaths in 2013. The EU notification rate was 0.44 cases per 100,000 population in 2013 which represented an 8.6% increase compared with 2012. A statistically significant increasing trend of listeriosis was reported in the European Union and European Economic Area (EU/EEA) over the period 2009–2013 (with 1,615, 1,663, 1,515, 1,644, 1,763 confirmed cases reported in 2009, 2010, 2011, 2012 and 2013, respectively (EFSA and ECDC, 2015)). The surveillance report from the European Centre for Disease Prevention and Control (ECDC) provides an overview of the epidemiological situation during the period 2010–2012 of the seven food- and waterborne diseases in the EU, including listeriosis. The notification rates of listeriosis increased rapidly by age in the older age groups (over 65 years). It was noted that male cases were predominant in groups over 45 years of age. Their risk of infection was twice as high as the risk for women in the same age group (ECDC, 2015). In 2013, a total of 12 *Listeria* outbreaks were reported by seven Member States. This was more than in previous years (eight and nine outbreaks in 2011 and 2012, respectively, EFSA and ECDC (2015)).

Three EFSA outsourcing activities are currently ongoing[4] under 'Closing gaps for performing a risk assessment on *L. monocytogenes* in RTE foods.' The first activity aims to perform a systematic review on *L. monocytogenes* in a wide range of RTE foods to gain knowledge on the available evidence on the presence of *L. monocytogenes* in RTE foods in the EU and the risk factors for contamination of RTE foods. In the second activity, a quantitative risk characterisation on *L. monocytogenes* in RTE foods, starting from the retail stage, will be developed to estimate the public health risks from consumption of various RTE food categories contaminated with *L. monocytogenes*. In the third activity, whole genome sequencing (WGS) will be applied to compare isolates from different compartments along the food chain and from humans.

The Panel on Biological Hazards (BIOHAZ Panel) is requested by EFSA to issue a Scientific Opinion on *L. monocytogenes* contamination of RTE foods and the risk for human health in the EU. In particular, the BIOHAZ Panel is requested:

- To summarise and critically evaluate the most recent information on *L. monocytogenes* in RTE foods, and in particular from the following sources: (a) EU-wide baseline survey and monitoring data and (b) the three ongoing EFSA outsourcing activities, i.e. (i) the presence of, and risk factors for, *L. monocytogenes* in RTE in the EU, (ii) an estimation of the public health risks from consumption of various RTE food categories contaminated with *L. monocytogenes*, and (iii) the comparison of isolates from different compartments along the food chain, and in humans using whole genome sequencing (ToR 1).

[2] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, p. 1–26.

[3] Commission Decision of 5 November 2010 concerning a financial contribution from the Union towards a coordinated monitoring programme on the prevalence of *Listeria monocytogenes* in certain ready-to-eat foods to be carried out in the Member States (2010/678/EU). OJ L 292, 10.11.2010, p. 40–54.

[4] These activities were ongoing at the moment of launching the mandate.

- To discuss and evaluate the factors related to the contamination in the food chain and the consumption patterns that may contribute to the reported trend of listeriosis incidence (rates) in the EU (ToR 2).

1.2. Interpretation of the ToR

The definition of RTE food in Commission Regulation (EC) No 2073/2005[5] on microbiological criteria for foodstuffs was used: '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 acceptable level microorganisms of concern.'

Trend is defined as a monthly change in the number of human listeriosis cases or human listeriosis incidence rates over time, not just a change of the incidence rates between the start and the end of the time period considered.

The focus of this Scientific Opinion is on the time period after the adoption of the previous Scientific Opinion of the BIOHAZ Panel at the end of 2007 (EFSA BIOHAZ Panel, 2008), i.e. 2008–2015. For the ToR 2, it was decided to consider the EU/EEA instead of the EU.

1.3. Additional information

1.3.1. Additional background information

Transmission routes of *Listeria monocytogenes* in RTE foods

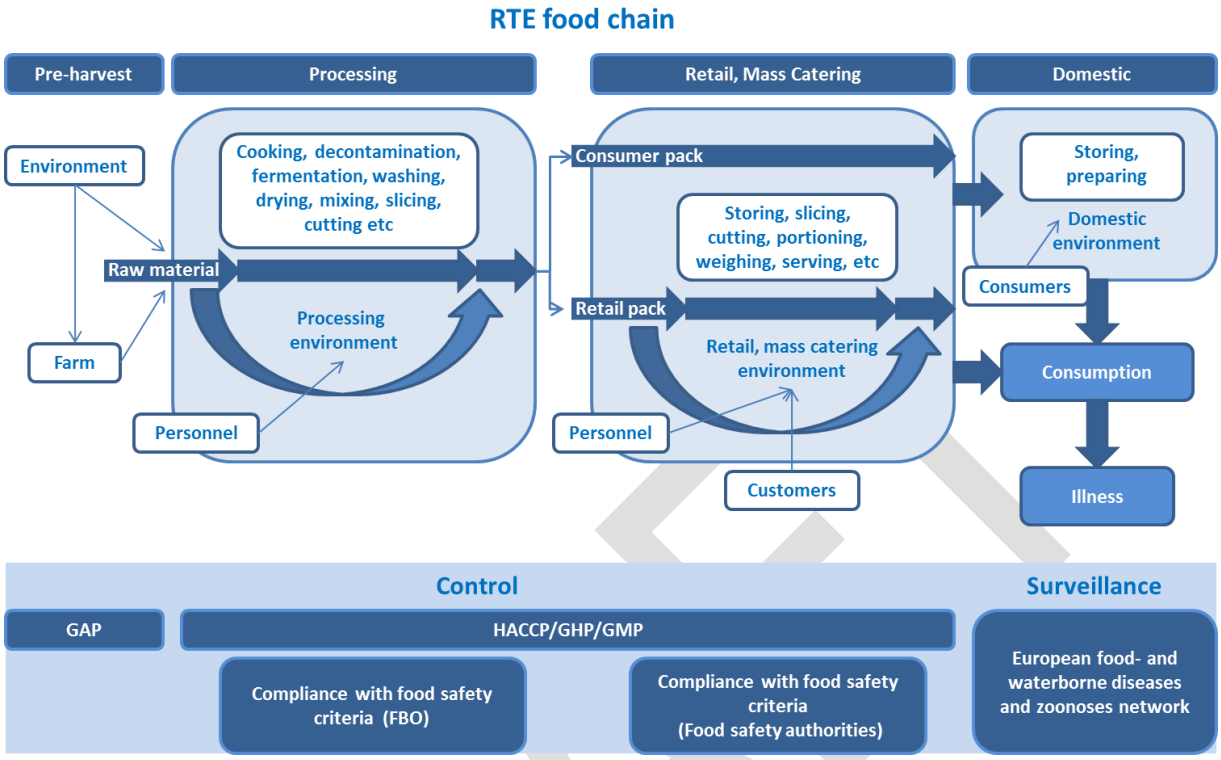
Listeria monocytogenes is a ubiquitous organism that is widely distributed in the environment. As shown in the overview of the transmission routes and the food safety control system of *L. monocytogenes* in RTE foods (Figure 1) there are a number of contamination routes whereby *L. monocytogenes* can enter the RTE food chain.

Soil and water are considered to be the primary sources of *L. monocytogenes* for transmission to plant material, feed, animals and the food chain (Linke et al., 2014). The pathogens can survive in the soil for months and even grow in favourable conditions (Dowe et al., 1997). The farm environment is frequently contaminated with *L. monocytogenes*, and is an important natural source for raw material contamination (Nightingale et al., 2004). The raw material from primary production entering the process is considered of great importance for the presence of the pathogen in the finished product. Indeed, the higher the pathogen concentration in the raw material, the more effective the control processes need to be in order to reduce concentrations to acceptable levels. In addition, the potential for contamination and persistence in the processing environment increases with increasing concentration of *L. monocytogenes* in the raw material entering the process.

RTE food processing may involve, among other processes, comminution, addition of flavourings, binders, extenders and emulsifiers, etc., addition of preservatives (e.g. lactate, sodium nitrite), decontamination (water, acid), heating (pasteurising, cooking, baking, boiling, steaming), curing, smoking (hot or cold), fermentation and drying. Most of these steps have the potential to reduce pathogen loads on the RTE food through microbial inactivation or inhibition of growth. The effectiveness of the control measures depends on the type of food and design of the process. In the case of a mild process (i.e. washing) the pathogen may survive while more intense or severe processes (i.e. sufficient heating) may lead to the elimination of the pathogen. RTE foods may also become re-contaminated during further processing and handling. In the latter case, increased handling leads to a higher probability of contamination. Sources of contamination may be food contact surfaces, processing machinery and workers. Contamination with *L. monocytogenes* after heat processing during further handling (cutting, slicing) is one of the most important occasions of contamination. This is due to the capability of *L. monocytogenes* to form biofilms which may result in enhanced resistance to sanitisers, disinfectants and antimicrobial agents.

[5] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, 26 pp.

RTE foods can be packed aerobically, under vacuum or modified atmosphere conditions. Packaging atmosphere can affect the growth of the pathogen during storage and hence the final risk. In addition, the amount of growth of *L. monocytogenes* can be affected by the assigned use-by date since this is likely to affect the storage time of the product.



FBO: food business operator; GAP: good agricultural practice; GHP: good hygiene practices; GMP: good manufacturing practices; HACCP: hazard analysis and critical control points; RTE: ready-to-eat.
Consumer pack: food packs that are not processed during retail; retail pack: food packs that are further processed (i.e. sliced) during retail.

Figure 1: Schematic overview of transmission routes and the control system of *Listeria monocytogenes* in ready-to-eat (RTE) foods

Following packaging, RTE foods are transported to retail or mass catering stores. Contamination of RTE food in packages that are opened and handled in retail stores (chubs, bricks, etc.) can also happen. According to Lakicevic and Nastasijevic (2017), food retail and mass catering 'establishments are very different from food processing plants. They are open to the public, with customers, sales people, employees, and deliveries coming into the establishment.' This may trigger 'the introduction of *L. monocytogenes* at various points and times of the day.' *Listeria monocytogenes* strains are regularly found and often widely distributed in retail facilities (Gombas et al., 2003; Pradhan et al., 2011). Retail practices may result in cross-contamination from one RTE product to another, through contamination from the retail environment, or from both (FSIS and FDA, 2013). Continued presence of *L. monocytogenes* in a particular environmental site (i.e. slicing machine) at retail can be a 'niche' that may facilitate continued cross-contamination of products from environmental sources. Survey studies reported that RTE deli meats handled at retail stores have, in general, higher contamination than pre-packaged products, indicating the possibility of cross-contamination at retail level (Gombas et al., 2003; Pradhan et al., 2011; FSIS and FDA, 2013).

Food can also become contaminated at the domestic level. Sources may be opened RTE packages that are often stored for extended periods in the home refrigerator or other niches in the kitchen. This suggestion is supported by isolations of *L. monocytogenes* from different kitchen environments (Evans and Redmond, 2016b).

Growth of *L. monocytogenes* 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 the environment (biofilms). RTE foods are a broad and diverse food category, some of which support

growth of *L. monocytogenes* and others that do not support growth or even result in microbial inactivation in specific storage and shelf life conditions. Factors affecting *L. monocytogenes* growth mainly include the product characteristics (pH, a_w, concentration of antimicrobials), storage temperature and time. The microstructure of the food matrix can also affect the growth by imposing physical constraints on microorganisms, by limiting the diffusion of essential nutrients and oxygen or by preventing the diffusion of metabolic products (Aspridou et al., 2014). The concentration of the pathogen at the time of consumption can be significantly affected by the lag phase duration. The latter is affected by the physiological state of the cells and is determined both by the growth environment (food) and the environment where cells were exposed before the contamination event (Robinson et al., 1998). The presence of competitive microflora is an additional factor that can affect growth (Mejlholm and Dalgaard, 2015). Indeed, several studies have shown that the presence of lactic acid bacteria have an inhibiting effect on *L. monocytogenes*. For example, in Norway a study found that indigenous lactic acid bacteria acted as a protective culture in cooked meat products that were sliced and either vacuum or gas packed (Bredholt et al., 1999). Winkowski et al. (1993) describe the inhibitory effect of *Lactobacillus bavaricus* in three beef foods. The effect is mainly based on the production of bacteriocin rather than acidification. Finally, growth of *L. monocytogenes* can also be strain dependent and contamination with a faster-growing strain can lead to higher concentration of the pathogen at the time of consumption (Whiting and Golden, 2002).

Control of *Listeria monocytogenes* in ready-to-eat foods

Many of the processes, routes and factors described above are monitored and controlled throughout the food chain (Figure 1). The public health risk from *L. monocytogenes* in RTE food also depends on the effectiveness of the control and monitoring procedures which include good agricultural practice at the farm stage and the hazard analysis and critical control points (HACCP) programme and good hygiene practices (GHP) at the processing and retail stages as well as sampling procedures to evaluate compliance with the FSC for *L. monocytogenes*. These are laid down in Commission Regulation (EC) No 2073/2005[6] on microbiological criteria for foodstuffs. This Regulation came into force in January 2006 and requires the following:

- In RTE products intended for infants and for special medical purposes *L. monocytogenes* must not be present in 25 g of sample;
- *L. monocytogenes* must not be present in levels exceeding 100 colony forming units per gram (CFU/g) during the shelf life of other RTE products; and
- In RTE foods that are able to support the growth of the bacterium, *L. monocytogenes* must not be present in 25 g of sample at the time of leaving the production plant; however, if the producer can demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 CFU/g throughout its shelf life, this criterion does not apply.

For more information, see Appendix A. In this Regulation RTE food is defined, as mentioned in Section 1.2.) as '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 acceptable level microorganisms of concern.'

There are several guidance documents available on *L. monocytogenes* in RTE foods. The guidance document on *L. monocytogenes* shelf life studies for RTE foods[7] aims to guide RTE producers in identifying the *L. monocytogenes*-associated risk in their RTE foods and to provide general principles on when and which shelf life studies are needed. It may also be used by competent authorities to verify the implementation of shelf life studies. The EU Reference Laboratory for *Listeria monocytogenes* (EURL *Lm*) technical guidance document on shelf life studies for *L. monocytogenes* in RTE foods[8] provides specialised laboratories with detailed and practical information on how to conduct shelf life studies (especially durability studies and challenge tests) for *L. monocytogenes* in RTE foods.

[6] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, p. 1–26.
[7] https://ec.europa.eu/food/sites/food/files/safety/docs/biosafety_fh_mc_guidance_document_lysteria.pdf
[8] https://ec.europa.eu/food/sites/food/files/safety/docs/biosafety_fh_mc_technical_guidance_document_listeria_in_rte_foods.pdf

The guidelines on sampling the food processing area and equipment for the detection of *L. monocytogenes*[9] describe sampling procedures to be performed by food business operators manufacturing RTE food which may pose an *L. monocytogenes* risk for public health in order to detect *L. monocytogenes* on the surfaces of RTE food processing areas and equipment.

Previous Scientific Opinion of the BIOHAZ Panel

The previous Scientific Opinion of the BIOHAZ Panel was prepared in response to a request from the European Commission to update the scientific literature from a former Opinion of the Scientific Committee on Veterinary Measures relating to Public Health on the *L. monocytogenes* risk related to RTE foods, and to provide scientific advice on different levels of *L. monocytogenes* in RTE foods and the related risk for human illness (EFSA BIOHAZ Panel, 2008). The Panel concluded that, after a general decline in the 1990s, the number of human listeriosis cases in Europe had increased since 2000. The disease was found to be associated with pregnancy, but it was predominantly associated with immuno-compromised persons among those over 60 years old. No routine methods permitted the differentiation between virulent and avirulent *L. monocytogenes* strains. The foods which could be associated with transmission of human listeriosis were mostly RTE foods that support *L. monocytogenes* growth. Surveys of foods had not only collected data on the prevalence and contamination levels of *L. monocytogenes* in different food types, but also revealed associations with other parameters including: food packaging type, preparation practices (e.g. the use of slicing machines for meat products), storage temperatures, the stage of sampling with respect to shelf life, the lack of an effective HACCP system, and the lack of education and training for food handlers. Growth of *L. monocytogenes* was pointed out to be a function of the type of food and the storage time and temperature. Storage temperature in retail and domestic refrigerators was found to vary significantly, especially for the latter. Application of microbiological criteria was considered as one of several management activities to ensure that RTE foods presented a low risk to public health. The Panel concluded that such criteria would assist the control of *L. monocytogenes* levels, e.g. absence in 25 g or ≤ 100 CFU/g at the point of consumption. The available risk assessments at that time had concluded that most human listeriosis cases were due to foods markedly above the latter limit. The most recent Codex document on microbiological criteria for *L. monocytogenes* in RTE foods suggested a zero tolerance throughout the shelf life of the RTE foods in which growth can occur. The Opinion raised a concern that the application of a zero tolerance criterion close to the end of shelf life could classify products as unsatisfactory, although they would be of low risk. An additional option proposed in the Codex document was to tolerate 100 CFU/g throughout the shelf life provided that the manufacturer is able to demonstrate that the product will not exceed this limit throughout the shelf life. For RTE foods that support *L. monocytogenes* growth, the Opinion stated that it is impossible to predict with a high degree of certainty that the level will or will not exceed 100 CFU/g during their shelf life. Thus, applying this option may result in accepting a probability that those foods with > 100 CFU/g will be consumed. The impact on public health would depend on whether levels markedly higher than 100 CFU/g were reached. The Opinion identified a need for more thorough investigations of sporadic and outbreak cases of human listeriosis, as well as for consumption data on RTE foods that support growth of *L. monocytogenes*, in order to better assess the risk and improve knowledge of the foods associated with human listeriosis. It was recommended that comparisons between studies (e.g. surveys) should only be made when similar sampling strategies had been applied and that studies should focus on RTE foods able to support *L. monocytogenes* growth. In addition to microbiological criteria the consistent application of GHP in combination with HACCP was stressed as important to minimise the initial contamination at manufacturing level, and/or to reduce the potential for *L. monocytogenes* growth. The integrity of the chill chain, especially at the domestic level, as well as advice on diets and food storage (particularly for the elderly) were identified as areas for improvement to reduce the risk of human listeriosis.

[9] https://ec.europa.eu/food/sites/food/files/safety/docs/biosafety_fh_mc_guidelines_on_sampling.pdf

Outsourcing activities under 'Closing gaps for performing a risk assessment on *L. monocytogenes* in RTE foods'

In 2014 EFSA decided to outsource three activities under 'Closing gaps for performing a risk assessment on *L. monocytogenes* in RTE foods':

- activity 1: an extensive literature search and study selection with data extraction on *L. monocytogenes* in a wide range of RTE foods;
- activity 2: a quantitative risk characterisation on *L. monocytogenes* in RTE foods, starting from the retail stage; and
- activity 3: the comparison of isolates from different compartments along the food chain, and in humans using whole genome sequencing (WGS) analysis.

The **first activity** had as general objective to perform an extensive literature search to describe:

- the occurrence and levels of contamination of *L. monocytogenes* in RTE foods (review question 1); and
- the risk factors for the *L. monocytogenes* contamination in different RTE foods (review question 2).

The contract resulting from a negotiated procedure was awarded to a consortium with the Institut de Recerca i Tecnologia Agroalimentàries (IRTA) as leader and the University of Cordoba (UCO) as partner (NP/EFSA/BIOCONTAM/2015/04 – CT1). A report was published as the outcome of this activity and will be referred to throughout this document as Jofré et al. (2016). This activity followed up the work of a former procurement containing a protocol that included the literature search strategy and study selection criteria (at level 1 relevance screening) used for both review questions (RC/EFSA/BIOCONTAM/2014/01).

The overall objective of the **second activity** was to provide EFSA with a quantitative risk characterisation of *L. monocytogenes* in various RTE food categories in the EU, starting from the retail stage. The contract resulting from an open call for tender was awarded to a consortium with UCO as leader and IRTA as partner (OC/EFSA/BIOCONTAM/2014/02 – CT1). A report was published as the outcome of this activity and will be referred to throughout this document as Pérez-Rodríguez et al. (2017). The specific objectives were:

- to carry out a search and critically review data and existing microbial risk assessments on listeriosis and *L. monocytogenes* in RTE foods (hazard identification);
- to determine the exposure of humans in the EU to *L. monocytogenes* from consumption of various RTE food categories (exposure assessment);
- to assess the potential for *L. monocytogenes* to cause illness in human populations (hazard characterisation/dose–response (DR)); and
- to apply an appropriate model, integrating exposure and DR models, in order to estimate the public health risks from consumption of various RTE food categories contaminated with *L. monocytogenes* (risk characterisation).

The **third activity** had as overall objective to compare *L. monocytogenes* isolates collected in the EU from RTE foods, compartments along the food chain and humans using WGS analysis. The contract resulting from an open call for tender was awarded to a consortium with the Statens Serum Institut (Copenhagen, Denmark) as leader with three partners (French Agency for Food, Environmental and Occupational Health and Safety (ANSES), Maisons-Alfort, France; Public Health England, London, United Kingdom and the University of Aberdeen, Aberdeen, United Kingdom) (OC/EFSA/BIOCONTAM/2014/01 – CT 1). A report was published as the outcome of this activity and will be referred to throughout this document as Møller Nielsen et al. (2017). The specific objectives were:

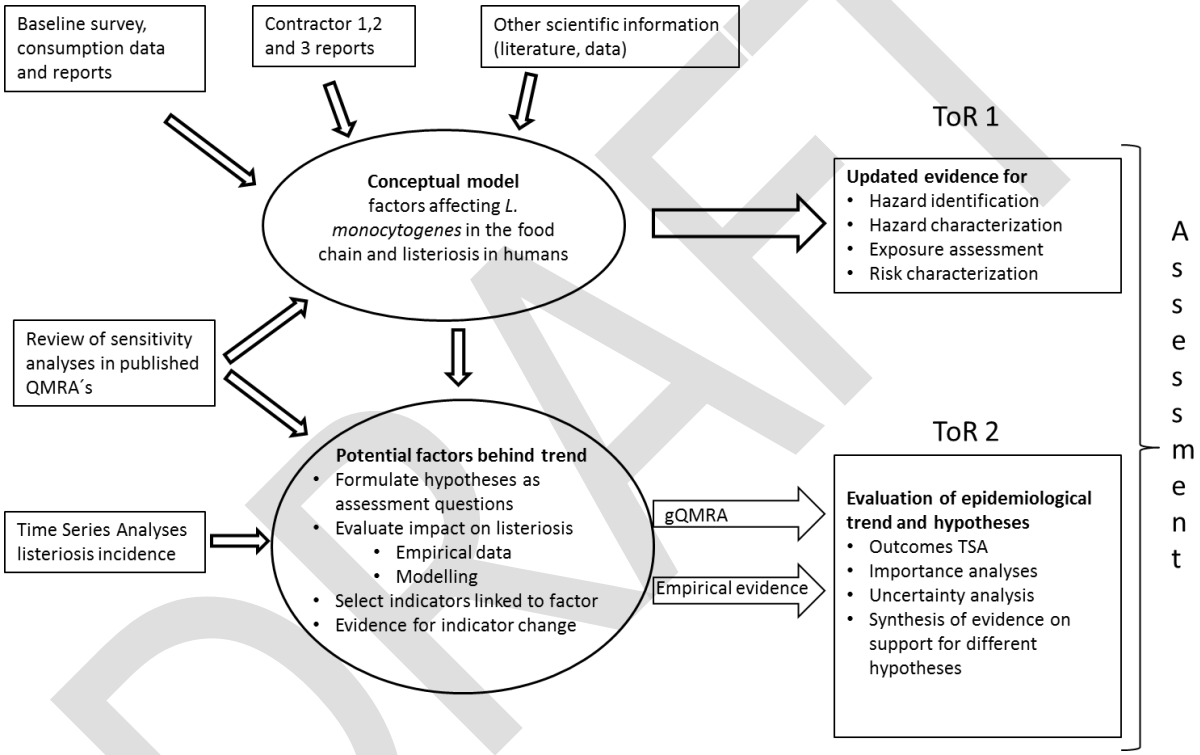
- to carry out the molecular characterisation of a selection of *L. monocytogenes* isolates from different sources, i.e. RTE foods, stages along the food chain (e.g. food-producing animals, food-processing environments) and humans, employing WGS analysis.
- to analyse the WGS typing data of the selected *L. monocytogenes* isolates with three goals:

- to explore the genetic diversity of *L. monocytogenes* within and between the different sources and human origin;
- to assess the epidemiological relationship of *L. monocytogenes* from the different sources and of human origin considering the genomic information and the metadata available for each isolate; and
- to identify the presence of putative markers conferring the potential to survive/multiply in the food chain and/or cause disease in humans (e.g. virulence and antimicrobial resistance).

- to perform a retrospective analysis of outbreak strains (i.e. using a subset of epidemiologically linked human and food isolates) to investigate the suitability of WGS as a tool in outbreak investigations.

1.3.2. Approach to answer the ToR

The approach to answer the ToRs is presented in Figure 2.



gQMRA: generic quantitative microbiological risk assessment; ToR: terms of reference; TSA: time series analyses.

Figure 2: Flow chart of the approach to answer the terms of reference

Terms of reference 1

The approach taken to answer to ToR 1 was to provide an update of the previous Scientific Opinion of the BIOHAZ Panel (EFSA BIOHAZ Panel, 2008) with a focus on new information, especially from the sources mentioned in the mandate. The new information was critically evaluated and summarised into descriptions of current knowledge so as to be able to support conclusions and to identify knowledge gaps. In addition, the contractors supplying the information were given feedback during their work to support their efforts and to ensure that useful information was obtained as input to the present Scientific Opinion.

Terms of reference 2

The approach taken to answer to ToR 2 was to analyse the trend of human listeriosis 'notification rates' (i.e. notified incidence rates) in the EU/EEA in detail and to evaluate key factors and hypotheses that may contribute to this trend. A time series analysis (TSA) of human listeriosis in the EU/EEA was

carried out at different levels of aggregation, e.g. aggregated by total confirmed cases, and disaggregated by age–gender groups. Based on the new information highlighted in the ToR 1, and other relevant information, a conceptual model of factors and processes of relevance for transmission of *L. monocytogenes* in the food chain and for the reported incidence rates of human illness via RTE foods was developed. The outcome of the TSA analysis, combined with the conceptual model, a review of sensitivity analyses from published risk assessments, and the reports of the three outsourcing activities, was the basis for identifying factors in the food chain to address in ToR 2 as possibly important drivers for *L. monocytogenes* contamination of RTE foods and reported human listeriosis illness. The identified factors/hypotheses were formulated as assessment questions (AQs)[10] and were then evaluated either qualitatively or quantitatively by combining evidence from risk assessment modelling, indicator data (i.e. empirical data linked to explanatory factors and that indicate any changes in the factor of interest), and the TSA. The partial food chain from retail to consumption was modelled and the influence of factors earlier in the chain was considered through their effects on prevalence and concentration at retail.

2. Data and methodologies

2.1. Data

2.1.1. Human data

ECDC data on cases of human listeriosis

Surveillance of human listeriosis focuses on severe invasive forms of *L. monocytogenes* infection, mostly manifested as septicaemia, meningitis or spontaneous abortion. The disease is reported by EU Member States and EEA countries in accordance with Decision No 1082/2013 on serious cross-border threats to health, repealing Decision No 2119/98/EC[11]. The cases are reported annually to The European Surveillance System (TESSy) in accordance with the EU case definition for listeriosis[12]. The number of reporting countries increased from 29 to 30 in 2013 when Croatia joined the EU and started to report data from 2012. Between 2008 and 2015, the national surveillance systems were comprehensive in 27 countries (28 from 2012). Partial population coverage was reported in Spain and Belgium throughout the whole eight-year period. The partial population coverage improved in Spain from 25% in 2008–2012 to 45% in 2015 (EFSA and ECDC, 2015). The human data are published annually in the EU summary reports[13] and are available in the interactive Surveillance Atlas[14] on the ECDC website. In addition, annual epidemiological reports are available on the ECDC website[15].

For the TSAs, monthly data on human listeriosis cases by country, age groups (<1, 1–4, 5–14, 15–24, 25–44, 45–64, 65–74, ≥ 75) and gender were extracted from TESSy for the period 2008–2015 (N = 15,026). Bulgaria reported only aggregated data for all years, and thus could not be included in the TSA dataset. Three countries (Croatia, Lithuania and Portugal) were excluded due to incomplete year-coverage of reported case-based data for the whole eight-year study period (72 cases excluded). Cases with missing data for age group, gender and/or month were excluded (N = 169). Cases under one year old were excluded from the TSA because they were mainly related to pregnancies and the reporting of mother–child pairs varied largely by countries (N = 633 cases). Finally, remaining cases reported as 'probable,' 'possible' or with 'unknown' classification were excluded (N = 150). The final

[10] According to the draft Guidance on Uncertainty in EFSA Scientific Assessment (EFSA Scientific Committee, 2016), an assessment question is 'a question to be addressed by an assessment. Assessment questions may be quantitative (estimation of a quantity) or categorical (e.g. yes/no questions). Many questions may usefully be divided into sub-questions for assessment.'

[11] Decision No 1082/2013/EC of the European Parliament and of the Council of 22 October 2013 on serious cross-border threats to health and repealing Decision No 2119/98/EC. OJ L 293, 5.11.2013, p. 1–15.

[12] Commission implementing Decision of 8 August 2012 amending Decision 2002/253/EC laying down case definitions for reporting communicable diseases to the Community network under the Decision No 2119/98/EC of the European Parliament and of the Council (2012/506/EU). OJ L 262, 29.9.2012, p. 1–57.

[13] http://www.efsa.europa.eu/en/zoonosesscdocs/zoonosescomsumrep

[14] http://ecdc.europa.eu/en/data-tools/atlas/Pages/atlas.aspx

[15] http://ecdc.europa.eu/en/publications/surveillance_reports/annual_epidemiological_report/Pages/epi_index.aspx

dataset for the TSA consisted of 14,002 confirmed human listeriosis cases for 2008–2015 from 24 EU Member States and two EEA countries (Iceland and Norway).

For serogroup–outcome (death/alive) analyses, case-based TESSy data from 2007 to 2015 were used. The reporting of serogroups by PCR typing was introduced to EU-level surveillance in 2012. Between 2012 and 2015, an increasing number of countries have moved from conventional serotyping to PCR-based serogrouping. To address this reporting change in the dataset, the serotypes were grouped under four serogroups following the published and accepted scheme (Doumith et al., 2004):

- 1/2a + 3a = IIa;
- 1/2b = IIb;
- 1/2c + 3c = IIc; and
- 4b + 4d,e = IVb.

The case numbers with serogroups IIb and IIc were relatively low which did not make it possible to perform meaningful analyses. As the serogroups IIa and IVb constituted 87% of all reported serogroups, the outcome analyses were performed with these two serogroups only. The final pooled dataset for 'outcome' and serogroup IIa and IVb analyses consisted of 3,308 confirmed cases from 15 countries (14 EU Member States and Norway).

Trends by serogroup were analysed for IIa and IVb over the period 2008–2015. Inclusion criteria required that a Member State had reported serotype or serogroup data throughout the whole study period. The trends of serogroups were described by year with a mean and 95% confidence interval (CI) for four Member States.

Data on food-borne outbreaks caused by *Listeria*

Within the framework of the EU Zoonoses Directive 2003/99/EC[16], the EU Member States are required to submit data on the occurrence of zoonoses, zoonotic agents, antimicrobial resistance and food-borne outbreaks. EFSA, in collaboration with ECDC, coordinates the collation and analysis of these data to produce the annual EU summary reports[17] which include data on food-borne outbreaks. The latter represents the most comprehensive set of data available at an EU level for assessing the burden of food-borne outbreaks in the EU/EEA and the related contributing risk factors. Data on 'strong evidence' food-borne outbreaks caused by *Listeria* from 2008 to 2015 were extracted from the EFSA zoonoses database. For these 'strong evidence' outbreaks, more detailed information is collected than for the 'weak evidence' food-borne outbreaks, including food vehicle and its origin, nature of evidence linking the outbreak cases to the food vehicle, extent of the outbreak, place of exposure, place of origin of the problem and contributory factors.

Eurostat data on European demographic statistics

The Statistical Office of the EU (Eurostat) collects data from EU Member States in relation to populations as of 1 January each year under Regulation 1260/2013[18] on European demographic statistics. The recommended definition is the 'usually resident population' and represents the number of inhabitants of a given area on 1 January of the year in question (or, in some cases, on 31 December of the previous year). However, the population provided by the countries can also be based either on data from the most recent census adjusted by the components of population change produced since the last census, or on population registers. Data were extracted from the 'Population on 1 January by age and gender' (demo_pjan[19]) database on 16 August 2016. Data were selected considering 'AGE' by selection of all ages (less than one year, 1 year, 2 years, 3 years, …, 99 years, open-ended age class), 'GEO' by selection of the EU Member States, 'SEX' by selecting males and

[16] Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC. OJ L 325, 12.12.2003, p. 31–40.
[17] http://www.efsa.europa.eu/en/zoonosesscdocs/zoonosescomsumrep
[18] Regulation (EU) No 1260/2013 of the European Parliament and of the Council of 20 November 2013 on European demographic statistics. OJ L 330/39, 10.12.2013.
[19] http://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=demo_pjan&lang=en

females and 'TIME' by selecting years 2008 to 2015. Then these data have been aggregated to derive the gender–age groups corresponding to those selected for the ECDC data on cases of human listeriosis (see above). The open-ended age class contains all the people aged more than the last single age for which a country can report. For example, if a country can provide data on its population by single year of age up to 94 years old, the open ended age class contains the population 95 years old and over. There were only open-ended age classes over 75 years reported and hence this did not have an impact on the aggregated data.

EU/EEA data on underlying conditions

- The number of adults (> 15 years) living with human immunodeficiency virus infection (HIV) in the EU/EEA was estimated by ECDC using their modelling tool[20] and HIV and acquired immune deficiency syndrome (HIV/AIDS) surveillance data on newly diagnosed cases through 2015 which is published in the annual surveillance reports[21] (Pharris et al., 2016). The number of women and men or persons within age groups has been estimated by ECDC for the purposes of this Scientific Opinion by applying the proportions of all cumulative cases diagnosed within the EU (i.e. proportions of males and females by age group 15–64 and ≥ 65 years) to the overall figure.
- Data on reported type-2 diabetes in the EU/EEA stratified by age from 20–79 in the years 2011, 2013 and 2015 has been provided by the International Diabetes Federation[22] (Brussels, Belgium).
- The absolute number of live births (births of children that showed any sign of life) was extracted from the 't_demo_fer' database[23] on 17 November 2016. The prevalence of pregnant women was derived by calculating the number of live births × 9/12.
- The Global Health Data Exchange website[24] was used to extract data on neoplasms, cirrhosis and other chronic liver diseases, chronic kidney disease, and HIV/AIDS in western Europe by gender and for the following age classes: < 5 years, 5–14 years, 15–49 years, 50–69 years and over 70 years old.

2.1.2. Data on *Listeria monocytogenes* contamination of ready-to-eat (RTE) foods

EU-wide baseline survey data

An EU-wide baseline survey (BLS) was conducted in 2010 and 2011 to estimate the EU prevalence (and contamination levels) of *L. monocytogenes* in three RTE food categories, in samples selected at random at retail level in accordance with Decision 2010/678/EU[25]: packaged (not frozen) smoked or gravad fish (3,053 samples), packaged heat-treated meat products (3,530 samples) and soft or semi-soft cheese (3,452 samples). The survey specifications defined particular subsets of food products to be sampled, specifically (i) RTE fish which were hot smoked or cold smoked or gravad, were not frozen, and were vacuum, or modified atmosphere, packaged; (ii) RTE meat products which had been subjected to heat treatment, and were then vacuum, or modified atmosphere, packaged; (iii) RTE soft or semi-soft cheese, excluding fresh cheese. This category includes smear-ripened, mould-ripened, brine-matured or otherwise ripened cheese, and concerns cheese made from raw, thermised or pasteurised milk of any animal species. The cheese could be packaged, or unpackaged at retail but packaged at the point of sale for the consumer. Only packaged and intact (sealed) packages, packaged by the manufacturer, were to be collected for sampling. However, in the case of cheese and

[20] https://ecdc.europa.eu/en/publications-data/hiv-modelling-tool
[21] http://ecdc.europa.eu/en/publications/surveillance_reports/hiv_sti_and_blood_borne_viruses/pages/hiv_aids_surveillance_in_europe.aspx
[22] http://www.idf.org/
[23] http://ec.europa.eu/eurostat/web/population-demography-migration-projections/births-fertitily-data/database
[24] http://www.healthdata.org/
[25] Commission Decision of 5 November 2010 concerning a financial contribution from the Union towards a coordinated monitoring programme on the prevalence of *Listeria monocytogenes* in certain ready-to-eat foods to be carried out in the Member States (2010/678/EU). OJ L 292, 10.11.2010, p. 40–54.

meat products, products packaged at the retail outlet could also be collected for sampling. A proportionate stratified sampling scheme was followed to allocate the number of samples to each Member State approximately according to the size of their human population. It should be noted that when reference is made in this Scientific Opinion to 'RTE fish,' 'RTE meat' and 'RTE cheese,' the above specifications apply.

Detailed information about the study can be found in two reports describing the results of this survey (EFSA, 2013, 2014). In the latter, multiple-factor analysis (generalised estimating equations) was used to investigate the statistical association between several factors on which information was gathered during the BLS, and two outcomes: prevalence of *L. monocytogenes* and proportion of samples with counts exceeding 100 CFU/g, in the surveyed fish and meat products.

EFSA monitoring data

The monitoring data collected by EFSA on *L. monocytogenes* in food originate from the reporting obligations of Member States under the EU Regulation on microbiological criteria (see Section 1.3.1.). It should be noted that, as stated by Boelaert et al. (2016),

> although the matrices sampled are harmonised and the sampling and analytical methods are harmonised to a certain extent, the sampling objectives, the place of sampling and the sampling frequency vary or are interpreted differently between Member States and according to food types. As such, these data are not comparable across Member States. The majority of these data are food chain control data (official monitoring) and are collected by the National Competent Authorities conducting investigations to verify whether food business operators implement correctly the legal framework of own-control programmes as well as the analyses in the framework of HACCP (industry monitoring) according to the General Food Law principles. Industry data are seldom reported to EFSA because of data ownership sensitivities. In essence, food chain control data are compliance checks and are collected with the aim to install an early warning and initiate control measures. In addition, the data sources are not transparently documented, as industry IT-based traceability solutions are currently not mandatory and companies may store data in arbitrary formats, including non-digital ones, as evidenced during food-borne disease outbreaks.

Thus, since information from different investigations is not necessarily directly comparable between Member States or for the same Member State across years, findings must be interpreted with care.

In the EU summary reports (e.g. EFSA and ECDC (2015)), the reported results of *L. monocytogenes* testing in RTE food samples are evaluated in accordance with the *L. monocytogenes* microbiological criteria indicated in Commission Regulation (EC) No 2073/2005 (≤ 100 CFU/g for RTE products on the market) applying certain assumptions, where appropriate. For many of the reported data, it was not evident whether the RTE food tested was able to support the growth of *L. monocytogenes* or not. For the non-compliance analysis of samples collected at processing, the criterion of absence in 25 g was applied, except for samples from hard cheese and fermented sausages (assumed to be unable to support the growth of *L. monocytogenes*), where the limit ≤ 100 CFU/g was applied. For samples collected at retail, the limit ≤ 100 CFU/g was applied, except for RTE products intended for infants and for special medical purposes, where the presence of *L. monocytogenes* must not be detected in 25 g of the sample. The results from qualitative examinations using the detection method have been used to analyse the compliance with the criterion of absence in 25 g of the sample, and the results from quantitative analyses using the enumeration method have been used to analyse compliance with the criterion ≤ 100 CFU /g.

EU Rapid Alert System for Food and Feed data

Commission Regulation (EU) No 16/2011[26] lays down the implementing measures for the requirements of Regulation (EC) No 178/2002 around the Rapid Alert System for Food and Feed (RASFF)[27]. This is established as a system facilitating the notification of food and feed safety alerts

[26] Commission Regulation (EU) No 16/2011 of 10 January 2011 laying down implementing measures for the Rapid alert system for food and feed. OJ L 6, 11.1.2011, p. 7–10.
[27] http://ec.europa.eu/food/safety/rasff/index_en.htm

among the competent authorities of Member States. RASFFs might typically deal with notification of food batches where sampling and analysis as a result of companies' own checks, border control, official control on the market, etc., has detected non-conformance with regard to the *L. monocytogenes* microbial criterion or other criteria; or where food batches have been implicated in illnesses. The RASFF system is primarily a communication facility enabling many food safety risks to be averted before they could be harmful to European consumers. It is not an epidemiological surveillance system but provides some understanding of the types of hazards typically detected in particular foods. It should be noted that RASFF notifications are not based on fully harmonised notification criteria and are not statistically representative, neither of the occurrence of *L. monocytogenes* in specific food products nor of the distribution of food-borne outbreaks associated with *L. monocytogenes* or a specific food. Moreover, it is important to note that information from different investigations is not necessarily directly comparable between Member States owing to differences in sampling strategies and the analytical methods applied and may not accurately represent the national situations across the EU. The purpose of using RASFF data for this assessment was to investigate the types and ranges of RTE foods where *L. monocytogenes* has been recovered during the period, to compare this in a qualitative manner with foods implicated in food-borne outbreaks, and to extract information on the concentrations of *L. monocytogenes* in these foods. For the purpose of this assessment, a search was conducted on 13 December 2016 of the RASFF database using as product category 'food' and the hazard '*Listeria monocytogenes*.' The search was restricted to the time period from 2008 onwards. The notifications were screened in duplicate and only those foods considered as RTE were included for further analysis.

The data for a selected number of RTE food categories were further analysed for the concentration of this pathogen. Only notifications with a reported concentration were considered and those notifications reporting a concentration range or only the presence of the pathogen were excluded from the analysis. For notifications providing concentrations of more than one sample the average value was used for the analysis.

Data from scientific literature and outsourcing activities

An extensive literature search was conducted in December 2015 by Jofré et al. (2016) to gather information on the occurrence and levels of contamination of *L. monocytogenes* in RTE foods (i.e. RTE foods, leafy greens and melons and traditional meat products) and risk factors for *L. monocytogenes* contamination of various RTE foods. The searches were done on SCI-EXPANDED and MEDLINE databases within the timespan 1990–2015. Relevance of the records was screened from the title and abstract (level 1), resulting in 1,448 unique records. After level 2 screening for eligibility, 308 records were identified as eligible for data extraction. Information was extracted about the study, RTE product (population) and analytical methodology, risk factors (exposure and comparators) and results (outcomes) about prevalence and concentration of *L. monocytogenes*. More information can be found in Jofré et al. (2016).

To assess the change in prevalence over time per food category, i.e. 'indicator data,' data were selected considering 'survey' as the aim of the study and 'retail' as the sampling location. In all food categories, the distribution of the prevalence values was asymmetric, with several outliers as well as extreme values. The high diversity in the type of products within each of the three major categories, the number of samples surveyed per study, the sampling locations in the farm-to-retail continuum and within the retail sub-sector (e.g. supermarkets, catering services, canteens, vendors, etc.) as well as in the duration of the survey (from one year up to 10 years), makes it difficult to draw conclusions on clear trends with time. For these reasons, the following selection criteria were applied to generate prevalence plots over time: (i) for surveys with duration greater than a year, the middle year was considered as the year of survey, (ii) different products were aggregated together into the major RTE food category, e.g. meat, seafood and dairy, (iii) the various sampling locations at retail were grouped, and (iv) only surveys were considered. This means that studies for testing the performance of in-house detection methods, or studies involving challenge testing that aimed to test the efficiency of a decontamination intervention in reducing *L. monocytogenes* numbers, were excluded.

For the analysis of the epidemiological relationship of *L. monocytogenes* isolates collected in the EU from RTE foods, compartments along the food chain and humans using WGS analysis, results from the third outsourcing activity by Møller Nielsen et al. (2017) were also considered.

2.1.3. Data on consumption of RTE foods

EFSA consumption data on RTE foods

The EFSA Comprehensive European Food Consumption Database[28] contains data on food consumption habits and patterns across the EU. It provides detailed information for a number of European countries in refined food categories and specific population groups. Summary food consumption statistics (chronic and acute) are available for each country, survey, age group (from infants to the elderly) and FoodEx1[29] food group (over 1,500) in g/day and g/kg bodyweight per day.

The consumption data for the three RTE food categories sampled in the EU-wide BLS were extracted from the database. More specifically FoodEx1 categories were used to identify eating occasions for semi-soft and soft cheese, cooked meat, sausage and pâté. As FoodEx1 was not detailed enough, the original national food descriptors were used to identify eating occasions for smoked and gravad fish. The same RTE foods in the three RTE food categories as considered by Pérez-Rodríguez et al. (2017) were selected.

Information related to the surveys included:

- country;
- survey;
- survey starting date and end date;
- total number of subjects; and
- total number of days for which consumption events were reported.

Summary statistics were reported for the following population strata and food groups:

- age class: 1–4, 5–14, 15–24, 25–44, 45–64, 65–74, ≥ 75 years old;
- gender; and
- food group: smoked fish, gravad fish, cooked meat, heat-treated sausages, pâté, soft and semi-soft cheese.

Food consumption summary statistics extracted from the Comprehensive Database:

- total number of eating occasions;
- total amount (g) consumed on all eating occasions;
- mean number of eating occasions per day in all days;
- mean, medium, 25th percentile and 75th percentile for the number of eating occasions per day in consuming days only; and
- mean, medium, 25th percentile and 75th percentile for the amount (g) per eating occasion in consuming days only.

Data were available from 23 Member States and 51 surveys. The most recent survey per Member State and age class was considered to estimate summary statistics. Thus data were considered from the 23 Member States and from 40 surveys. The survey starting date ranged from 1997 to 2012. The mean of the mean, medium, 25th percentile, and 75th percentile amount (g) per eating occasion on consuming days only was calculated for the various food groups and by gender and age class. Also the mean of the mean number of eating occasions per day on all days was calculated for the various food groups by gender and age class. The latter was multiplied by 365 to estimate the mean yearly number of eating occasions for the various food groups. By considering the population size in the EU/EEA in 2015, the total number of servings per year per age group and gender was derived. The reason for including surveys prior to the period of interest was the consideration that for descriptive

[28] http://www.efsa.europa.eu/en/food-consumption/comprehensive-database
[29] Hierarchical system based on 20 main food categories that are further divided into subgroups up to a maximum of four levels.

purposes it was more important to capture variation among countries than to capture the exact time period of interest.

Variation in consumption over time in the elderly population (≥ 65 years old) was estimated based on surveys from countries reporting more than once during the 1997–2015 time period, i.e. Denmark, Finland, the Netherlands and Sweden. This information was used as 'indicator data' for any change in consumption. Mean serving sizes and the number of servings per year were estimated for each survey as described above, and any differences were presented as differences in mean serving size or number of servings per country for the two occasions (survey year).

Food and Agriculture Organization data on smoked salmon consumption in the EU

In order to get a rough estimate of the possible smoked salmon consumption in the EU for a recent period (2003–2013), data from the Food and Agriculture Organization of the United Nations (FAO) were accessed, through the application FishstatJ and the workspace FAO Fishery and Aquaculture Statistics.[30] Production, import and export data (weight in tonnes) were obtained for the EU countries for the years 2003–2013 (production data were not available for all countries). Subsequently, a calculation was made in which production and import weights were added for each year/country and export weights were subtracted from this sum.

2.1.4. Literature review on consumer behaviour including storage and handling

For information relating to consumer behaviour when dealing with food, a literature search was done in the Web of Science database on 13 September 2016. No language or time restrictions were applied. The search was done in the topics field of the scientific publications using the keywords: (behaviour* OR behavior* OR handling OR attitude* OR kitchen* OR refrigerator* OR home* OR domestic OR practice* OR hygiene) AND (consumer* OR adult* OR elderly OR senior* OR people OR women OR men OR children OR toddler* OR vulnerable OR pregnant OR pregnancy) AND ((food NEAR safety) OR listeria OR listeriosis OR monocytogenes). A total of 2,747 records were retrieved, 2,740 after removal of duplicate records. In total, 218 references were considered potentially relevant. These records were further screened and relevant articles, based on geography (Europe) and scope of the article (behaviour and storage temperatures in relation to gender, age, socioeconomic factors) were selected. This process resulted in 32 records being considered as relevant and were reviewed in detail.

For information relating to the storage temperature of foods, a literature search was done in the Scopus database on 6 December 2016. No language or time restrictions were applied. The search was done in the topics field of the scientific publications using the keywords: temperature AND refrigerator. A total of 698 records were retrieved, from which 35 references were considered relevant and reviewed in detail. Additional records were added based on expert knowledge on relevant literature.

2.1.5. Surveillance of human listeriosis

A short questionnaire was sent to the nominated public health contact points for listeriosis and *Listeria* isolates in the European Food- and Waterborne Diseases and Zoonoses network (FWD-Net) on changes in diagnostic practices and national surveillance systems for listeriosis in 2008–2015. Separate questionnaires were addressed to the epidemiologists and microbiologists in 31 EU/EEA countries. The response rate was 61% for epidemiologists and 42% for microbiologists. A descriptive analysis of responses was made and the potential impact on EU-level data was assessed by reflecting the case counts between 2008 and 2015 in countries with notable changes in their national surveillance systems.

[30] FAO. ©2016. Fishery and Aquaculture Statistics. Global Fisheries commodities production and trade 1976-2013 (FishstatJ). In: FAO Fisheries and Aquaculture Department [online or CD-ROM]. Rome. Updated 2016. http://www.fao.org/fishery/statistics/software/fishstatj/en

2.1.6. Review of quantitative microbiological risk assessment (QMRA) outputs

A systematic review was conducted (with a literature search done in March 2016) by Pérez-Rodríguez et al. (2017) to retrieve existing microbiological risk assessments on listeriosis and *L. monocytogenes* in RTE foods. The searches were done in bibliographic databases (Scopus, Web of Science and MEDLINE databases) and other web sources. There were no time restrictions and no language restriction was imposed *a priori*. Relevance of the records was screened from the title and abstract (level 1), resulting in 122 records. After level 2 screening for eligibility, 47 records remained (40 scientific articles and seven reports, all published between 1996 and 2015). Information was extracted covering aspects such as: scope, approach and technical aspects, hazard characterisation/dose–response information, exposure assessment and risk characterisation. More information can be found in Pérez-Rodríguez et al. (2017). The 47 records were reviewed for identification of the factors (i.e. prevalence, storage time and temperature, slicing, product formulation, packaging type, serving size, and consumer susceptibility) and their levels (when applicable) that influence the risk of listeriosis, associated with the consumption of different RTE foods, namely deli meats, seafood, dairy products and fresh cut salads.

2.2. Methodologies

2.2.1. Time series analysis (TSA) of human listeriosis trends, 2008–2015

The 2008–2015 *L. monocytogenes* total number of confirmed cases reported to ECDC was first evaluated using an aggregated analysis. As analysis of aggregated trends may hide existing trends within subgroups of the EU/EEA population – and to gain further insights – data were also analysed in a disaggregated analysis by age–gender subgroups. Additional variables to create even finer subgroups were not available.

As explained in Section 2.1.1, data from countries which had not reported data for the whole study period 2008–2015 were excluded. In R, data on populations and cases were merged, providing a 'long' aggregated dataset, with the following variables: 'date,' 'gender,' 'age group,' 'cases,' and 'population.' Data for which the information on 'age group' and 'gender' was reported as 'unknown' were dropped, as well as records for which months had 'NULL' as an attribute. This resulted in a total of 14,002 listeriosis cases. The data were then transformed into a 'wide' dataset, with the following variables: 'year,' 'month,' 'cases' for gender–age group combinations, and 'population' for gender–age group combinations. The following 14 gender–age group combinations were used: 1–4, 5–14, 15–24, 25–44, 45–64, 65–74, ≥ 75 years old, for both males and females.

Aggregated time series analysis

The aggregate *L. monocytogenes* series from January 2008–December 2015 is a short time series that exhibits changing dynamics of the outcome variable (i.e. the *L. monocytogenes* incidence rates). Given these properties, specifying a proper time series model is difficult. As a first step, a series of autoregressive integrated moving average (ARIMA) models were attempted to address the serial correlation and the seasonal components. An ARIMA (1,0,0) (0,1,1) was successfully fit and had white noise residuals. However, these models had parameter estimates at the borderline of being non-stationary. This means that they are unstable and not good candidates for inference or forecasting. Another alternative to consider here would be a Bai and Perron (Bai and Perron, 1998; Bai and Perron, 2003) change point regression model. When this model was examined, after accounting for the serial correlation, the optimal number of change points was zero, indicating that there is not a single change point in the time series process. A change point model is also more restrictive than a dynamic linear model (DLM), since it only allows k = finite change points. The DLM allows the mean/variance estimate to change each time period, so it is the least restrictive approach possible.

Furthermore, a changing trend in time was observed. In this case, the commonly accepted approach is to use a trend and seasonal decomposition approach (West and Harrison, 1997; Petris et al., 2009). The simplest model in this class examines a random walk with seasonal dummy variables, α_{jt}, for the months. This model is made up of two equations, one for the observed data and the other for the

latent random walk. Let L_t be the *Listeria* time series at time t. The random walk model with seasonal effects with mean m_t is then

$$L_t = m_t + v_t \quad v_t \sim N(0, V) \qquad \text{Equation 1}$$

$$m_t = m_{t-1} + \sum_{j=1}^{12} \alpha_{jt} + w_t, \quad w_t \sim N(0, W) \qquad \text{Equation 2}$$

where the errors v_t and w_t are uncorrelated. The key pieces being estimated here are the variances V and W, since these explain how much of the variance is in each component of the model. In this v_t is the error term on the measurement or observation equation that maps the observed data to the state equation. The w_t are the errors for the state or dynamic equation that characterises the dynamics of the model. The V and W terms are the variances on these mean zero, normal, error terms.

The alternative model to see if there is a trend is a local linear growth model (i.e. a second order trend model). This allows for a second order trend that can capture shifts in trend beyond the random walk model. In contrast with a first order model which is linear (slope is either up or down) a second order model allows an inflection point (up/down versus down/up), allowing a good level of complexity in the shape. This model allows for a time-varying slope for m_t. The equations for this model extend those given earlier to include a trend term β_t. This extends the earlier equations as follows:

$$L_t = m_t + v_t \quad v_t \sim N(0, V) \qquad \text{Equation 3}$$

$$m_t = m_{t-1} + \beta_t + \sum_{j=1}^{12} \alpha_{jt} + w_t, \quad w_t \sim N(0, W) \qquad \text{Equation 4}$$

$$\beta_t = \beta_{t-1} + u_t, \quad u_t \sim N(0, U) \qquad \text{Equation 5}$$

The analyses were conducted with the 'dlm' package (Petris, 2010) in R version 3.3.3 (Ihaka and Gentleman, 1996; R Core Team, 2016), allowing the implementation of DLMs.

Disaggregated age–gender groups time series analysis

To obtain a visual impression of the evolution of reported confirmed human listeriosis incidence rates, by age and gender, smoothed trend lines based on local regressions were computed (Cleveland et al., 1992).

For modelling of the disaggregated cases data, the low number of cases in certain age and gender subgroups, and the non-normality of the residuals did not allow for the use of a dynamic linear modelling approach, as was the case for the aggregated data. Instead the trends in the subgroups required the use of a model that allowed the handling of small numbers of observed counts at the same time as being able to deal with autocorrelation of the counts. An appropriate model for such a situation is a Poisson autoregressive model (PAR(p)) (Brandt and Williams, 2001). This model is based on an extended Kalman filter for the count process and allows for analysis of cyclical properties (e.g. autocorrelation).

The equation estimated for the PAR(p) models is

$$y_t = rho \times y_{t-1} + (1 - rho) \times \exp(constant + trendcoefficient \times t + \log(population)) \qquad \text{Equation 6}$$

where y_t is the count at time t and y_{t-1} is the lagged count, with t indicating time, *rho* expressing the autocorrelation coefficient for a one month lag model (for some of the count time series a second lag was also needed to account for second order serial correlation). The remaining term in the exp() expresses the constant, the time trend in logarithms and the population offset. The PAR(p) model described by (Brandt and Williams, 2001) and the R-code to handle such models had to be adapted in order to include log(population) offset for the subgroup populations, an offset being an adjustment term with a parameter estimate (for log(*population*)) constrained to 1.

The equation for the trends computation is then the component

$$\exp(constant + trendcoefficient \times t + \log(population)) \qquad \text{Equation 7}$$

More details on the basis of this model can be found in Brandt and Williams (2001).

The analyses were conducted with an adapted version of the 'pest' commands (Brandt and Williams, 2001) for R version 3.3.3 (Ihaka and Gentleman, 1996; R Core Team, 2016), enabling the implementation of autoregressive Poisson models. Adaptations to the code were required to produce some results specific to this study. For comparing the incidence rates of specific groups (e.g. males

and females of an age group) the package 'epitools' (Aragon, 2012) in R version 3.3.3 (Ihaka and Gentleman, 1996; R Core Team, 2016) was used. The R-code of the TSA is available in Appendix B.

2.2.2. Comparison of rates

For comparison of rates (case fatality or incidence rates), the 'rateratio' function of the 'epitools' package (Aragon, 2012) for R version 3.3.3 (R Core Team, 2016) was used, allowing the calculation of the rate ratio, confidence intervals and *p* values, by median-unbiased estimation (mid-*p* method). This method compares rates by using a test of independence. An alpha level of 0.1 was applied without multiple comparisons correction. The multiple comparison procedure applies to all possible comparisons among the factor level case fatality rates (CFRs).

2.2.3. Assessment questions

As illustrated in the approach flow chart (Figure 2), a number of factors that may contribute to any change in the number of cases or incidence rates of human listeriosis were identified based on the conceptual model, the TSA, the review of sensitivity analyses in published QMRAs, and the reports of the three outsourcing activities (Jofré et al., 2016; Møller Nielsen et al., 2017; Pérez-Rodríguez et al., 2017). To be able to evaluate the potential contribution of these factors on the incidence rates of human listeriosis a number of AQs were formulated. In addition to the change in human listeriosis, differences in incidence rate levels between population groups were also considered to be important for the analysis. Since formulation of AQs is a potential source of uncertainty (EFSA Scientific Committee, 2016) special care was taken to formulate them with the support of a technical expert. Factors are separated based on whether they are related to the host (AQ1.1–1.2), the food (AQ2.1–2.4), the national surveillance systems (AQ3.1), or the bacterium (AQ4.1):

- **AQ1.1:** What contribution did any change in the population size (i.e. the number) of the elderly and/or susceptible people make to the change in cases of human listeriosis in the EU/EEA in the time period 2008–2015?
- **AQ1.2:** What contribution did any change in 'underlying condition rate' make to the change in incidence rates of human listeriosis in the EU/EEA in the time period 2008–2015?
- **AQ2.1:** What contribution did any change in *L. monocytogenes* prevalence in RTE food at retail level make to the change of human listeriosis incidence rates in the EU/EEA in the time period 2008–2015?
- **AQ2.2:** What contribution did any change in *L. monocytogenes* concentration in RTE food at retail level make to the change of human listeriosis incidence rates in the EU/EEA in the time period 2008–2015?
- **AQ2.3:** What contribution did any change in storage conditions (temperature, time) after retail (i.e. consumer phase) make to the change of human listeriosis incidence rates in the EU/EEA in the time period 2008–2015?
- **AQ2.4:** What contribution did any change in consumption (serving size and frequency) make to the change of human listeriosis incidence rates in the EU/EEA in the time period 2008–2015?
- **AQ3.1:** What contribution did any change of (improved) surveillance make to the change of human listeriosis incidence rates in the EU/EEA in the time period 2008–2015?
- **AQ4.1:** What contribution did any change in virulence make to the change of human listeriosis incidence rates in the group of interest in the EU/EEA in the time period 2008–2015?

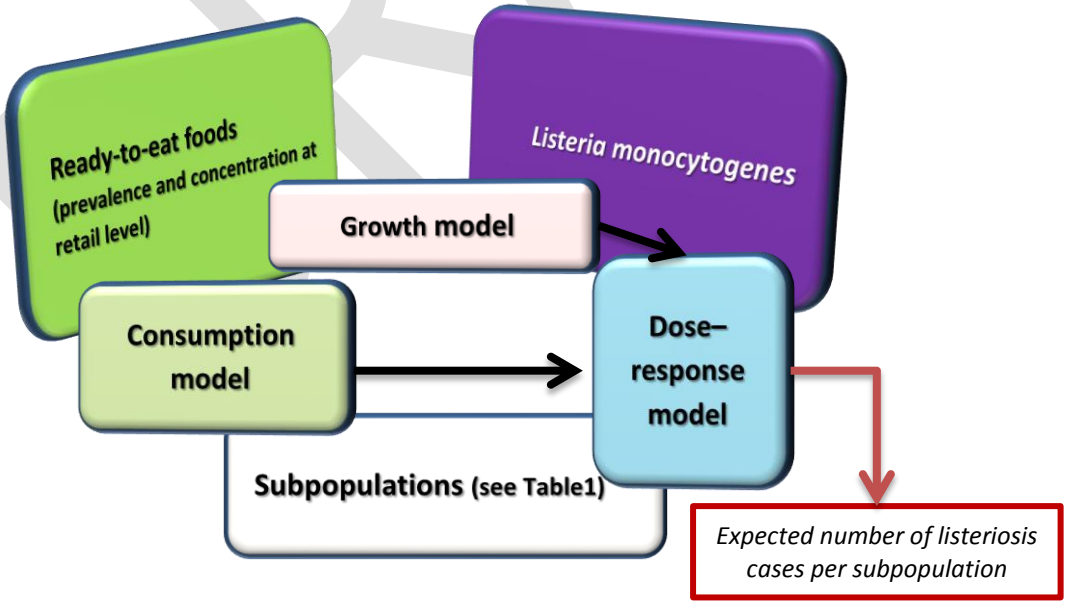
The AQs were evaluated stepwise. First, an importance analysis identified the most important factors that may have an impact by using the gQMRA model (See Section 2.2.4). The second step was to evaluate empirical evidence, or indicator data to investigate the support for a change in the factor during the time period. In the third step, a synthesis was made of the TSA, the importance analyses, the empirical evidence and the uncertainty analyses. Based on the outcome of this evaluation, conclusions, with uncertainties described, were drawn on the impact of the different factors on the human listeriosis incidence rates and data gaps were identified.

2.2.4. *Listeria monocytogenes* generic QMRA (gQMRA) model

In order to address the identified factors/hypothesis explaining the increase of human listeriosis incidence rates in the EU/EEA a quantitative microbiological risk assessment model, referred to as the *L. monocytogenes* generic quantitative microbiological risk assessment (gQMRA) model, was developed by the working group (Figure 3) upon the model developed by Pérez-Rodríguez et al. (2017). The gQMRA model presents the following adaptations and improvements:

- Since the TSA addressed the listeriosis trend in 14 age–gender groups, the gQMRA model had to be adapted to address all these groups. The model by Pérez-Rodríguez et al. (2017) considered three groups: ≥ 65 years old, pregnant women and < 65 years old. Therefore, input data related to age–gender groups had to be developed, i.e. dose–response parameters, and consumption data.
- The estimation of exposure was used to assess a new DR model with parameters for the 14 age–gender groups, which was developed mainly based on epidemiological data on human listeriosis in the EU/EEA.
- The data on initial *L. monocytogenes* concentration in RTE foods was modified by also using US data to evaluate the effects of initial concentration and growth.
- Implementation of the model in R to allow for a higher number of iterations (millions of iterations) and therefore more stability of the model outputs (model convergence).
- The model is generic in the sense that the model allows the inclusion of more or fewer RTE food categories and subpopulations without changing the R code. As the inputs are provided in structured Microsoft Excel tables, the code automatically interprets the number of RTE food categories and the number of subpopulations.
- The R code and model implementation allow an expanded evaluation of uncertainty when the uncertainty about the inputs are available.
- Full inclusion of the variability related to the DR model.

The gQMRA model predicts consumer exposure based on the initial contamination level at retail of a variety of RTE foods, and the potential growth before consumption. The probability of a consumer being infected and developing listeriosis is then predicted by applying a DR model.



The gQMRA model is constructed around three main elements; food, population and hazard. It includes three models: consumption model, growth model and dose–response model. The overlapping of the model boxes with the main element boxes indicates that the model takes into account one or several factors characterising the food, the populations or the hazard.

Figure 3: *Listeria monocytogenes* generic quantitative microbiological risk assessment (gQMRA) model

More than 70% of cases of listeriosis occur in individuals with recognised underlying diseases such as liver disease, cancer and diabetes and would ideally need to be considered in the risk assessment model (Goulet et al., 2012). However, the lack of reliable data on the distribution of human listeriosis cases for the different underlying conditions groups as in the Goulet et al. (2012) study prompted the working group to apply another approach based on epidemiological data available in the EU/EEA using the same 14 subpopulations defined by age and gender as in the TSA. The distribution of the number of human listeriosis cases within these subpopulations is presented in Table 1 combined with their relative risk.

The input data of the gQMRA model and additional information related to the gQMRA assessment can be found in Appendix C.

Table 1: The distribution of the number of invasive human listeriosis cases in the EU/EEA (2008–2015) within the 14 subpopulations and estimated relative risks

Subpopulations	Population in EU/EEA (2008–2015)[(a)]	Proportions of subpopulations in EU/EEA	Listeriosis cases in EU/EEA (2008–2015)[(b)]	Relative risk[(c)]
Female 1–4 yo	9,981,292	0.021	49	0.17
Male 1–4 yo	10,507,387	0.022	62	0.20
Female 5–14 yo	24,769,674	0.052	52	0.07
Male 5–14 yo	26,071,451	0.054	51	0.07
Female 15–24 yo	27,917,371	0.058	209	0.26
Male 15–24 yo	29,107,545	0.061	72	0.08
Female 25–44 yo	67,013,021	0.140	1,067	0.54
Male 25–44 yo	68,019,328	0.142	351	0.18
Female 45–64 yo	65,803,889	0.137	1,219	0.63
Male 45–64 yo	63,791,535	0.133	2,001	1.07
Female 65–74 yo	24,249,576	0.051	1,328	1.87
Male 65–74 yo	20,921,720	0.044	2,142	3.50
Female ≥ 75 yo	25,539,929	0.053	2,537	3.40
Male ≥ 75 yo	15,476,863	0.032	2,862	6.33
Total population	**479,170,581**	**1**	**14,002**	**1**

yo: years old.

(a): The average of the yearly population figures during the time period 2008–2015 was used.

(b): Based on the final dataset for the TSA consisting of 14,002 confirmed human listeriosis cases for 2008–2015 from 24 EU Member States and two EEA countries (Iceland and Norway).

(c): Ratio of the incidence rate observed in one subpopulation to the incidence rate observed in the total population. A relative risk (RR) > 1 means that listeriosis is more likely to occur in the subpopulation than in the total population. A RR < 1 means that listeriosis is less likely to occur in the subpopulation than in the total population. RR = 1 means that there is no difference in the risk between the subpopulation and the total population.

Ready-to-eat foods

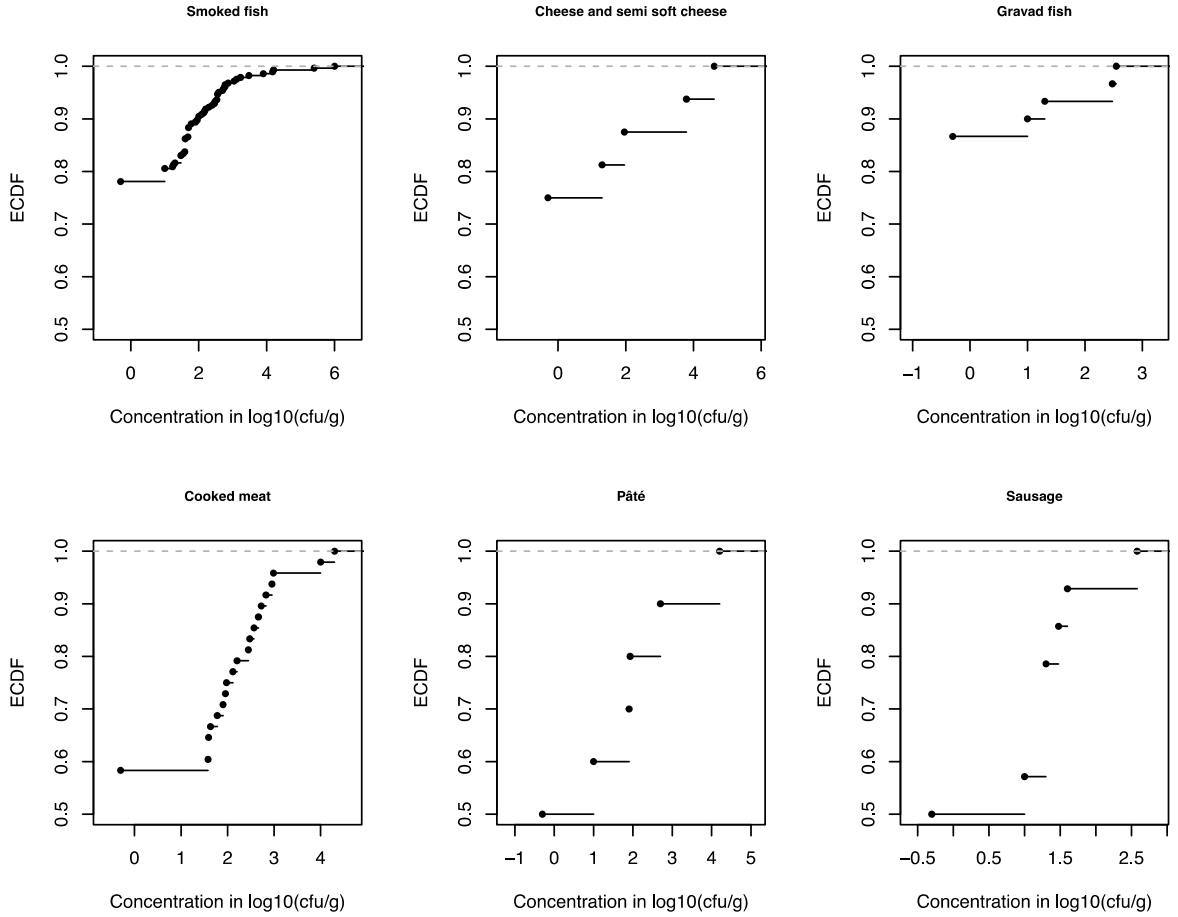
The seven RTE food subcategories considered in the gQMRA model are:

- Cold-smoked fish;
- Hot-smoked fish;
- Gravad fish;
- Cooked meat;
- Sausage;
- Pâté; and
- Soft and semi-soft cheese.

The gQMRA model starts at the retail level. The prevalence of contamination by *L. monocytogenes* is taken from the BLS data (see Section 2.1.2) (EFSA, 2013, 2014).

Good quality enumeration data for *Listeria monocytogenes* in RTE foods are rare. In the EU, the BLS (EFSA, 2013, 2014) provided the best available data on the concentration of *L. monocytogenes* in

certain RTE foods. The data were checked and are summarised in Figure 4. In the current model, for initial concentration only, data for cold-smoked and hot-smoked fish were combined, under the simplifying assumption that the two types of smoked fish have the same distribution of the initial concentration. It should be noted that in the BLS, enumerations were carried out at the end of shelf life of the RTE foods. For fish the enumerations were also done at the time of sampling and these data were used.



For better visibility, different scales of the x-axis were used. The empirical cumulative distribution function (ECDF) is a step function that jumps up by 1/n at each of the n data points. Its value at any specified value of the measured variable is the fraction of observations of the measured variable that are less than or equal to the specified value. Example: for pâté, curves show that concentration has a probability of 90% to be less or equal to 3 $\log_{10}$ CFU/g.

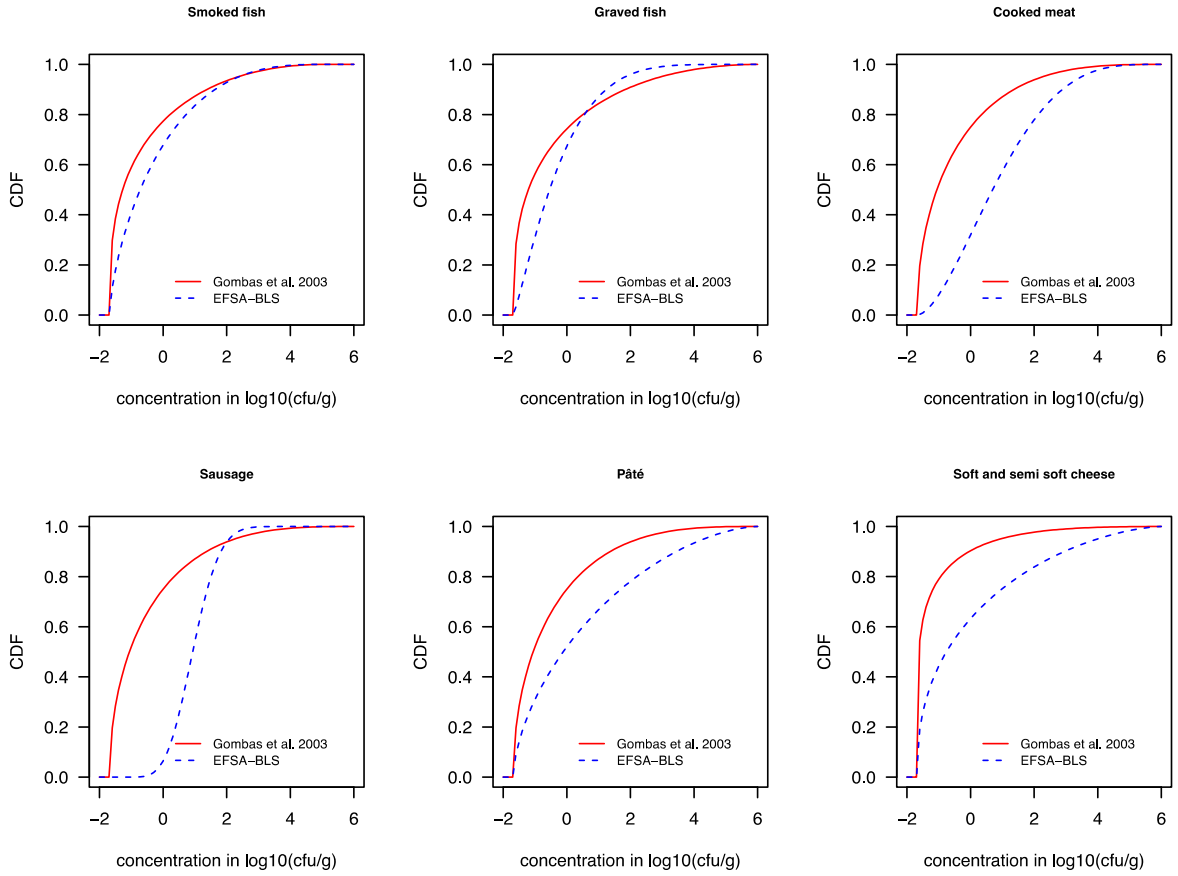
Figure 4: Empirical cumulative distribution function of *L. monocytogenes* concentrations per RTE food category based on BLS data (EFSA, 2013, 2014)

Listeria monocytogenes concentrations (at decimal logarithm scale) in RTE food were modelled using beta-general distributions with a minimum equal to -1.69 and maximum equal to 6.1. The two other (shape) parameters of the food-specific beta-general distributions (α and β) were estimated using a maximum likelihood estimation algorithm implemented in the 'mle' function ('stats4' package in R version 3.3.3 (Ihaka and Gentleman, 1996; R Core Team, 2016)). Positive samples without enumeration were assumed to have a concentration less than 10 CFU/g. The cumulative distribution functions (CDFs) of the used beta-general distributions are presented in Figure 5.

A similar study was conducted in the United States of America. Eight categories of RTE foods were collected over 14 to 23 months from retail markets in Maryland and northern California. The product categories included luncheon meats, deli salads, fresh soft 'Hispanic-style' cheese, bagged salads, blue-veined and soft mould-ripened cheese, smoked seafood, and seafood salads (Gombas et al., 2003). In order to compare the BLS and US data, it was assumed that luncheon meat sampled in the USA has the same initial concentration distribution as cooked meat, sausage and pâté sampled in the

EU. Likewise, the concentration for blue-veined and mould-ripened cheese in the US study are considered equivalent to soft and semi-soft cheese as sampled in the EU. Gravad fish was not included in the US study and therefore the samples from smoked fish sampled in the USA were used instead. Using the assumptions above and the same approach for fitting a cumulative distribution function, the BLS data and the US data were compared (Figure 5).

As shown in Figure 5, for meat products and cheese the BLS data resulted in a CDF that indicates a higher frequency of high concentrations compared with those obtained with Gombas et al. (2003) data. This difference could be explained, partially, by the fact that the enumerations were carried out at the end of shelf life of the RTE foods in the BLS (except for fish samples, which were analysed at sampling and at the end of shelf life) whereas in the Gombas et al. (2003) survey quantification was carried out directly after the sampling. For smoked fish, the product most similar in the BLS and US surveys, the concentration beta distributions based on BLS survey and Gombas et al. (2003) data had very similar CDFs. Gravad fish was not identified in Gombas et al. (2003); therefore, the presented CDF corresponds to smoked fish. Overall, the concentrations in gravad fish are lower than the concentrations observed in smoked fish.



The cumulative distribution function is the probability that the concentration will take a value less than or equal to a specific concentration. Example: the blue dashed curve shows that for smoked fish, the concentration has a probability of around 90% to be less or equal to 2 $\log_{10}$ CFU/g.

Figure 5: Fitted cumulative distribution functions of *L. monocytogenes* concentrations per RTE food subcategory obtained from the US data (Gombas et al. (2003)) and the BLS data

Faced with this uncertainty on the distribution of initial *L. monocytogenes* concentrations, it was decided to consider three options:

- Option 1. Use only the distributions estimated with BLS data.
- Option 2. Use only the distributions estimated with US data (Gombas et al., 2003).

- Option 3. Use fish distribution from BLS data, and meat and cheese distributions from US data (Gombas et al., 2003).

Option 3 was considered the best and was used as the baseline for the gQMRA model.

Consumption model

In this model, the average portion size (mass of RTE food ingested per meal) per RTE food category and subpopulation as well as the total number of eating occasions per year (TEO) were estimated from the EFSA consumption database and are presented in Section 3.3.3.

Growth model

Listeria monocytogenes can multiply at refrigeration temperatures. The exponential growth rate (EGR) can vary between the different RTE food categories. In order to capture this variability a review of available data on the *L. monocytogenes* EGR in different foods was carried out by Pérez-Rodríguez et al. (2017). The output of this review has been summarised using probability distributions of the EGR estimated at 5°C for the different RTE food categories in the risk assessment. It was assumed that the EGR at 5°C is log-normally distributed. Moreover the packaging conditions, i.e. reduced oxygen packaging (ROP; including both vacuum and modified atmosphere packaging) versus normal packaging, were considered as a factor modifying the growth potential of *L. monocytogenes* in all seven RTE food subcategories, except in soft and semi-soft cheese. Therefore the growth has been estimated for 13 subcategories/packaging conditions. The proportion of RTE food categories that are ROP packed was estimated using data collected during the BLS survey.

The EGR at a specific temperature T is derived using this simplified secondary model, with $Tmin$=−1.18°C:

$$EGR(T) = EGR(5°C) \times \left(\frac{T-T_{min}}{5-T_{min}}\right)^2 \quad \text{Equation 8}$$

The temperature (T) of the consumer refrigerator was assumed normally distributed with a mean equal to 5.9°C and a standard deviation of 2.9°C (Derens-Bertheau et al., 2015).

We are assuming in this model that the consumer stores the different RTE food categories at the same temperature.

The final concentration $C(t)$ at the end of the storage time (t) at temperature T is calculated using the following equation:

$$C(t) = \frac{C_{max}}{\left(\frac{C_{max}}{C(0)}-1\right)\times\left(exp(-EGR(T)\times t)\right)} \quad \text{Equation 9}$$

Where C_{max} is the maximum concentration or maximum population density (MPD) and $C(0)$ is the initial concentration before storage. This primary growth model does not consider a lag time. The possible growth between purchasing and storage in the refrigerator was ignored; because first no accurate data are available to consider this step and second the transportation to the laboratory after sampling may include part of this growth potential. The latter, however, is unlikely for the BLS samples as these samples had to be transported in refrigerated containers and had to be kept at between 2 and 8°C. Those received at a temperature higher than 8°C were rejected, unless the temperature at retail was higher than 8°C (EFSA, 2013).

The time of storage (t) was calculated following three steps:

- Step 1: determination of the remaining shelf life; in considering data observed within the BLS. The remaining shelf life of an RTE food at the time of its purchase was assumed to follow an exponential distribution which has a single parameter. This parameter was estimated for all seven RTE food subcategories and per type of packaging (ROP versus normal).
- Step 2: it was assumed that the RTE food can be consumed any time from immediately after purchase up to and beyond the remaining shelf life of the product (10% more) but more frequently consumers will consume the food after having stored it for a period of time equal to 0.30 of the remaining shelf life. This variability, as assumed by the working group members, was modelled with a beta-pert distribution with a minimum, mode and maximum

equal to 0, 0.30 and 1.1 respectively (this variable was named proportion of remaining shelf life, psl).

- Step 3: for each iteration of the model, the storage time is derived by multiplying together the two values obtained by sampling the respective distributions of psl and of the remaining shelf life.

Distribution of doses in 'generic' ready-to-eat food

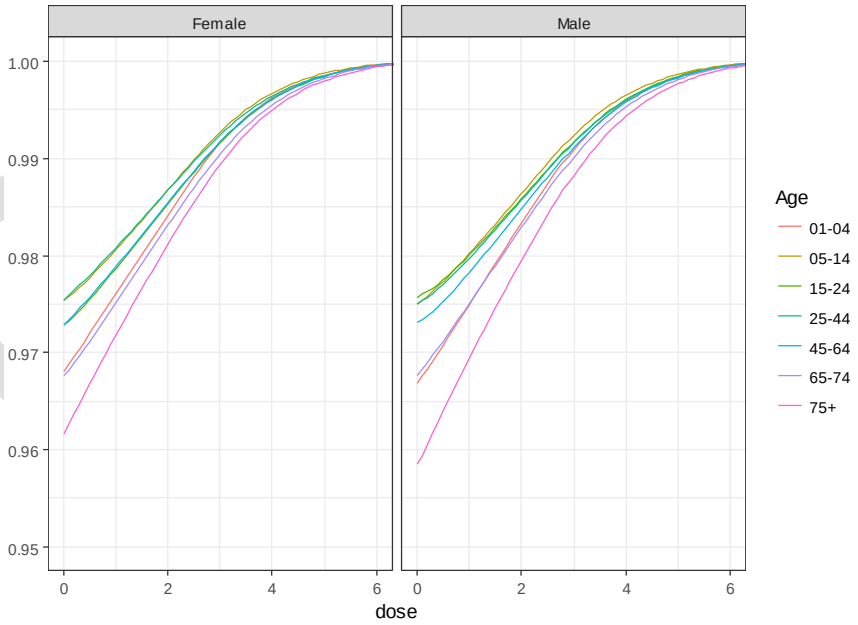
The concentration at the time of consumption, $C(t)$, was assessed for the 13 RTE food subcategories/packaging conditions. For each of these, one million iterations were performed to assess a specific $C(t)$ distribution. The expected exposure dose (λ) distribution was assessed for each RTE food subcategory/packaging condition in each of the considered age–gender groups as follows:

$$\lambda = 10^{C(t)} \times PS \qquad \text{Equation 10}$$

where PS is the portion size in g.

An overall expected exposure dose distribution in generic RTE food was assessed from those obtained for each of 13 RTE food subcategories/packaging conditions by weighting each category by its relative frequency of consumption (number of eating occasions for a RTE category/number of eating occasions for all the RTE food categories) in each of the considered subpopulations (age–gender groups). In the same way, an overall prevalence was estimated.

From the simulation model, the distribution of the concentration at time of consumption, i.e. the dose, is obtained for each of the 14 subpopulations for each of the three options of the initial concentration. Figure 6 shows the dose distributions using option 3, the baseline option. The starting points of the cumulative dose distributions are the overall proportion of non-contaminated RTE food for each of the 14 subpopulations (i.e. 1-Prevalence of contaminated RTE food). The differences in the overall prevalence are explained by the differences in the consumption patterns between the subpopulations. The lowest curves, for male and female, are obtained with the age category 'above 75 years old' meaning that these two populations are the most exposed to *L. monocytogenes* (Figure 6).



The y-axis represents the cumulative distribution function. This is the probability that the concentration will take a value less than or equal to a specific concentration. Example: the curve in the male population 'above 75 years old' shows that the concentration in the generic RTE food has a probability of around 98% to be less than or equal to 2 $\log_{10}$ CFU/g. Option 3: using fish products distribution from EU BLS data, and meat and cheese distributions from US data (Gombas et al., 2003).

Figure 6: Example of simulated doses distribution ($\log_{10}$ CFU of *L. monocytogenes* per eating occasions) in generic ready-to-eat (RTE) food based on using option 3 for the initial concentration of *L. monocytogenes* in the seven RTE food subcategories considered

Dose–response model

The DR model used in the gQMRA was assessed using the approach described in Pouillot et al. (2015). This model is a log-normal exponential model; given an expected dose λ (number of *L. monocytogenes* CFU per serving) the probability of illness is derived as follows:

$$P_{ill}(\lambda) = 1 - \exp(-r\lambda) \qquad \text{Equation 11}$$

$$with\ log10(r) \sim Normal(\mu, \sigma)$$

The *r* parameter, which represents the probability that one single CFU will survive the different barriers and multiply in a favourable site of infection, depends on the characteristics of the host and the strain of *L. monocytogenes*. By its definition, *r* is variable. To capture this variability, *r* was assumed to be log-normally distributed. The log-normal distribution is described by two parameters; μ and σ (mean and standard deviation). As epidemiological data show significant differences in incidence rate of human listeriosis between categories of age and gender, it was decided to estimate the mean of the log-normal distribution of *r* for each of the 14 populations. With parsimony, the standard deviation was assumed to be the same for each subpopulation: in this way, it characterises the intra-subpopulation variability of *r*.

To estimate the 14 means of *r*, we used as exposure the output of the exposure model (Figure 3), the average of the annual observed cases of human listeriosis per subpopulation between 2008 and 2011 and the TEO per subpopulation. This reference period is used because it corresponds to the period of data consumption collection and covers the period of the BLS. Estimating *r* consists in solving the following equation which has a single unknown value (mean of *r*):

$$Cases = TEO \times \left(1 - \int_{\lambda=0}^{\infty} \int_{r=0}^{1} \exp(-r \times \lambda)\, f(\lambda) g(r) d\lambda\, dr\right) \qquad \text{Equation 12}$$

Where $f(\lambda)$ and $g(r)$ are two probability distribution functions describing the variability of the expected doses (output of the gQMRA model) and the *r* parameter of the exponential model respectively. The standard deviation of $g(r)$ is considered constant and equal to 1.62 as in Pouillot et al. (2015). The equation is solved for each of the 14 subpopulations. Table 2 gives the estimated mean of $\log_{10}(r)$ for each of them.

Table 2: Estimated means of the *r* parameter estimated by the baseline gQMRA model for the 14 subpopulation groups

Subpopulations	Geometric mean of *r*
Female 1–4 yo	2.67E-15
Male 1–4 yo	3.41E-15
Female 5–14 yo	1.21E-15
Male 5–14 yo	9.89E-16
Female 15–24 yo	4.73E-15
Male 15–24 yo	9.20E-16
Female 25–44 yo	9.44E-15
Male 25–44 yo	1.72E-15
Female 45–64 yo	8.30E-15
Male 45–64 yo	9.02E-15
Female 65–74 yo	1.99E-14
Male 65–74 yo	2.75E-14
Female ≥ 75 yo	2.91E-14
Male ≥ 75 yo	2.91E-14

yo: years old. Option 3: Fish distribution using the BLS data, and meat and cheese distributions from US data (Gombas et al., 2003).

Expected number of human listeriosis cases per subpopulation

The different parts of the model were combined to estimate the number of human listeriosis cases in R version 3.3.3 (Ihaka and Gentleman, 1996; R Core Team, 2016). The code is available in Appendix C.

Importance analysis

An importance analysis was carried out to evaluate the potential for the different factors identified in the AQs to contribute to the change of listeriosis incidence rate. This analysis shows how much change in the factor is needed to explain different fractions of the observed change in listeriosis incidence rates. A comparison of this information with any empirical data on changes in the factor during the time period would indicate the extent of the contribution from the factor on the observed trend.

The importance analysis was carried out by running the gQMRA model with different value ranges for the following input parameters:

- maximum population density of *L. monocytogenes* in RTE foods;
- time of storage at consumer level: mode and maximum of the proportion of the remaining shelf life;
- temperature of consumer refrigerator during storage: mean; and
- initial concentration of *L. monocytogenes* in RTE foods: set of data (EU versus US).

For the evaluated factors it was found that the relative impacts on the outputs of the model were relatively close for all subpopulations so results were presented for the whole population.

2.3. Uncertainty

Based on the draft EFSA guidance on uncertainty (EFSA Scientific Committee, 2016), and the mandate, special attention was given to: (i) the interpretation of the ToRs, i.e. framing of the mandate and the AQs, (ii) identifying sources of uncertainty, and (iii), their impact on the outcome of the assessment. Focus of the uncertainty assessment was on ToR 2, i.e. uncertainty associated with the gQMRA model, the TSA and indicator data, on trying to assess the combined effects on uncertainties in answering the AQs. The identified assumptions and other sources of uncertainty were listed for those.

3. Assessment

New information and evidence related to factors important for *L. monocytogenes* contamination in the food chain and for the reported incidence rates of human illness are summarised in this section under the headings of a common risk assessment which makes up the response to ToR 1. A detailed analysis of the trends in human listeriosis incidence rates (2008–2015) is presented in Section 3.5, and an evaluation of potential contributing factors using different risk assessment approaches and models in Section 3.6, which together are the response to ToR 2.

3.1. Evidence for hazard identification

3.1.1. Introduction to the species *L. monocytogenes*

Several new species of the genus *Listeria* have been described during the last decade and the genus *Listeria* now consists of 17 distinct species (Orsi and Wiedmann, 2016). Most of the new species were isolated from rural sources and decaying material. Among all *Listeria* species, *L. monocytogenes* is still the only important agent from a human health perspective. The oral route is the central mechanism of exposure both for animals and humans and it is estimated that 99% of all human cases of listeriosis are food-borne (Orsi et al., 2011). *Listeria monocytogenes* is isolated from a variety of biotic and abiotic sources, the environment and foods. Both raw material contamination and cross-contamination during food processing may have an effect on prevalence and the concentration of *L. monocytogenes* in the final product. Concerning exposure of consumers, contamination of raw materials could be critical in cases where low-processed foods are produced and processing conditions are not efficient enough to reduce or eliminate the bacterium from the final product. In that context, cattle (cows and ewes) suffering from mastitis could be of notice since *L. monocytogenes* may be shed into the raw milk at very high numbers for extended periods of time and especially for on-farm dairies that often process such milk without any, or with uncontrolled, heat treatment (Wagner et al., 2005). Other examples could be low-processed fish products and a variety of foods of non-animal origin. In all

those cases it is possible that the contaminated raw material leads to a contamination of the food processing environment (FPE) and from there to the contamination of the final product. The most important route of contamination is considered to be via FPEs. *Listeria monocytogenes* is transmitted to food via introduction from environmental sources outside the processing facility (incoming raw materials, animals, soil, dust and water) into the FPE. Temporal breakdown in hygiene barrier efficiency such as during phases of reconstruction may trigger this and may also lead to a persistent colonisation of an FPE which can be seen as an intermediate step in transmission from the original habitat to the food being processed (Reij et al., 2004). Having colonised an FPE, *L. monocytogenes* may spread throughout the facility via aerosols, personnel, food workflows, and contaminated contact materials possibly leading to persistence if sanitation procedures are insufficient (Alali and Schaffner, 2013). FPEs often display a multitude of compartments, presenting challenges for efficient cleaning and disinfection. The problem is triggered by other factors such as inappropriate design of equipment, niche adaptation and biofilm formation that may lead to persistence of the bacterium (Carpentier and Cerf, 2011).

3.1.2. Epidemiology of human listeriosis in the EU/EEA

The notification rate of invasive listeriosis has increased between 2008 and 2015, from 0.30 to 0.46 cases per 100,000 population (Table 3). The notification rate is the closest estimate to a population-based incidence rate in the EU/EEA. The number of case reports increased by 60% from 1,381 confirmed cases reported in 2008 to 2,206 cases in 2015. Most listeriosis cases are sporadic and over 98% of human invasive *L. monocytogenes* infections are acquired domestically and most travel-related cases have acquired the infection within the EU/EEA. In 2015, 270 deaths were reported, which was the highest annual number of deaths reported due to listeriosis since 2008. The overall CFR was 17.7% in 2015 (EFSA and ECDC, 2016). The reported cases of confirmed human listeriosis and notification rates in the EU/EEA by country and year are provided in Appendix D. Estimates of under-reporting and under-ascertainment are lower than for many other pathogens (Haagsma et al., 2013), probably due to the severity of listeriosis, and factors around 1.7 to 2 have been reported in Canada, the USA and the UK (Mead et al., 1999; Adak et al., 2002; Thomas et al., 2013b).

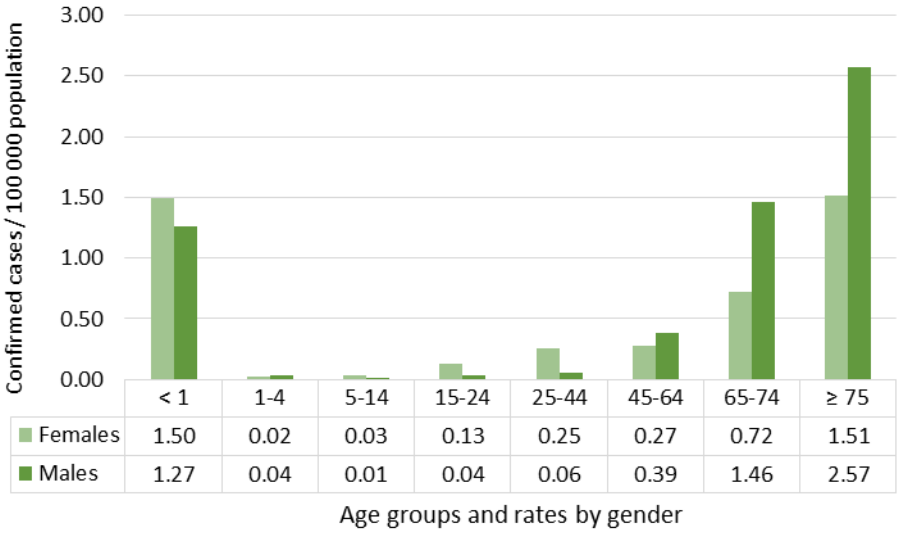
Table 3: Reported and published human cases of confirmed human listeriosis, related deaths and case fatality rates in the EU/EEA, 2008–2015

Year	Confirmed cases	Notification rate[(a)]	Cases with outcome data (% of confirmed cases)	Deaths	CFR (%; 95% CI)[(j)]
2008[(b)]	1,381	0.30	653 (47.3%)	134	20.5% (17–24)
2009[(c)]	1,645	0.36	757 (46.0%)	126	16.6% (14–19)
2010[(d)]	1,601	0.35	1,063 (66.3%)	181	17.0% (15–19)
2011[(e)]	1,476	0.32	1,054 (71.4%)	134	12.7% (11–15)
2012[(f)]	1,642	0.41	1,112 (67.7%)	198	17.8% (16–20)
2013[(g)]	1,763	0.44	1,228 (69.7%)	191	15.6% (14–18)
2014[(h)]	2,161	0.52	1,401 (64.8%)	210	15.0% (13–17)
2015[(i)]	2,206	0.46	1,524 (69.1%)	270	17.7% (16–20)

CFR: case fatality rate; CI: confidence interval.
(a): notified incidence rate.
(b): 2008 report: http://www.efsa.europa.eu/en/efsajournal/pub/1496.
(c): 2009 report: https://www.efsa.europa.eu/en/efsajournal/pub/2090.
(d): 2010 report: https://www.efsa.europa.eu/en/efsajournal/pub/2597.
(e): 2011 report: https://www.efsa.europa.eu/en/efsajournal/pub/3129.
(f): 2012 report: https://www.efsa.europa.eu/en/efsajournal/pub/3547.
(g): 2013 report: https://www.efsa.europa.eu/en/efsajournal/pub/3991.
(h): 2014 report: https://www.efsa.europa.eu/en/efsajournal/pub/4329.
(i): 2015 report: https://www.efsa.europa.eu/en/efsajournal/pub/4634.
(j): Aggregated estimate for reporting countries of case fatality (% of cases with known outcome), CI estimated in this Scientific Opinion.

The highest notification rates are commonly seen in the elderly, over 65 years old, and in children under 1 year of age.[31] The rate for males was double that for females in the age group 65–74 years in 2015 (Figure 7). In the age group 65–74 years, the rate for males was over 140 times higher than for males in the age group 5–14, while the respective rate ratio was 24 for females.

In addition to old age and increased susceptibility due to underlying conditions, medical practices and medications have been hypothesised as risk factors for human listeriosis (ACMSF, 2009b). Of special interest are treatments with proton pump inhibitors (PPI); it has been suggested that they influence susceptibility to several enteric pathogens, e.g. *Campylobacter*, *Salmonella*, and *Listeria* (Bouwknegt et al., 2014). PPI increase the gastric pH, encourage growth of the gut microflora, increase bacterial translocation and alter various immunomodulatory and anti-inflammatory effects (Bavishi and DuPont, 2011; Bouwknegt et al., 2014). A case–control study investigated the association between the use of PPI and the risk of non-pregnancy-associated listeriosis using Danish registry data (Jensen et al., 2017). The authors reported a temporal association between increased susceptibility to listeriosis and the use of PPI with an adjusted odds ratio (OR) of 2.81 (95% CI, 2.14–3.69). Based on the adjusted OR the population-attributable fraction of listeriosis due to current PPI usage was estimated at 8.3%. The OR increased with decreasing age, which might indicate a higher relative impact for people with a lower baseline risk (Jensen et al., 2017).



	< 1	1-4	5-14	15-24	25-44	45-64	65-74	≥ 75
Females	1.50	0.02	0.03	0.13	0.25	0.27	0.72	1.51
Males	1.27	0.04	0.01	0.04	0.06	0.39	1.46	2.57

Source: Data from The European Surveillance System – TESSy, provided by Austria, Belgium, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom, and released by ECDC.

Figure 7: Number of confirmed human listeriosis cases/100,000 population by age group and gender in the EU/EEA in 2015

Lethal *L. monocytogenes* infections particularly affect the population > 45 years old, with average annual CFRs ranging from 16.0% among 65–74-year-old males to 22.5% among females over 75 years old (Table 4). There were no significant differences in CFR between genders for all age groups except for the age group 21–44 where case fatality was almost four times higher for males than for females.

[31] http://ecdc.europa.eu/en/healthtopics/listeriosis/annual-surveillance-data/Pages/annual-surveillance-data.aspx.

Table 4: Mean annual case fatality rates with 95% confidence intervals by age group and gender in the EU/EEA, 2008–2015

Age group (years)	Males (N = 4,753)		Females (N = 3,837)	
	Mean CFR (%)	95% CI	Mean CFR (%)	95% CI
< 1	12.7	[8.5–16.9]	11.6	[8.0–15.2]
1–20	7.6	[1.8–13.5]	5.4	[1.2–9.7]
21–44	12.6	[9.4–15.7]	3.4	[1.7–5.1]
45–64	17.7	[15.4–20.1]	16.1	[13.3–18.8]
65–74	16.0	[13.5–18.5]	19.3	[16.3–22.4]
≥ 75	20.3	[18.3–22.4]	22.5	[20.0–25.0]

Source: Data from The European Surveillance System – TESSy, provided by Austria, Belgium, Croatia, Cyprus, Czech Republic, Estonia, France, Germany, Greece, Hungary, Ireland, Latvia, Lithuania, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, United Kingdom, and released by ECDC.
CFR: case fatality rate, CI: confidence interval.

3.1.3. Pregnancy-associated human listeriosis cases

The variable for reporting pregnancy-associated cases (Yes/No/UNK) was introduced in 2010 for 2009 data. Therefore, the time period for assessing the proportion of pregnancy-associated cases is 2009–2015. All pregnancy-associated cases were reported within the three age groups: < 1 year; 15–24 years and 25–44 years old (Table 5). As reporting of pregnancy-related cases varies by country (i.e. some countries report only a mother, only a child, or both) data for children under one year of age is also presented for the sake of completeness. The proportion of unknown information has been, on average, 44% over the years for females in the combined age group 15–44 years and thus the known pregnancy-associated cases account for about 56% of the reported females in this age group (N = 1,083). After the introduction of a new variable, the first two reporting years tend to be more unstable before the reporting routine has developed.

In the age group 15–24 years, the pregnancy-associated case proportions remained at about the same level until 2014–2015 when the proportion appears to have increased from 50% in 2013 to 75% in 2015. No marked increase was seen in the age group 25–44 years. The proportion of pregnancy-associated cases of the total cases reported in TESSy has ranged from 8% to 14% over the years.

Table 5: Number of cases and percentage pregnancy-associated in females by selected age groups and years reported in the EU/EEA, 2009–2015

Year	Age group (years)		
	< 1	**15–24**	**25–44**
	Number of cases (% pregnancy-associated)	Number of cases (% pregnancy-associated)	Number of cases (% pregnancy-associated)
2009	27 (40.7)	24 (50.0)	94 (35.1)
2010	70 (90.0)	14 (14.3)	73 (35.6)
2011	33 (87.9)	18 (55.6)	80 (58.8)
2012	29 (72.4)	18 (55.6)	96 (46.9)
2013	32 (84.4)	14 (50.0)	90 (57.8)
2014	41 (80.5)	21 (66.7)	114 (52.6)
2015	19 (78.9)	24 (75.0)	89 (60.7)
Total	251 (79.3)	133 (54.9)	636 (49.8)

Source: Data from The European Surveillance System – TESSy, provided by Austria, Estonia, France, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Poland, Romania, Slovenia, Sweden, United Kingdom, and released by ECDC.

3.1.4. Reported food-borne listeriosis outbreaks

Data reported in the zoonoses database on occurrence of 'strong-evidence' food-borne outbreaks caused by *Listeria* (2008–2015) at EU/EEA level can be found in Appendix E. A summary is provided in Table 6. A total of 37 strong-evidence food-borne outbreaks were reported with 525 human cases (i.e. less than 4% of total reported cases during the period), 182 hospitalisations and 37 deaths. The 'dairy' food category was responsible for four of these outbreaks causing 44 cases, while 'fish and seafood' and 'meat and meat products' food categories were responsible for 7 and 11 of these

outbreaks causing 40 and 126 cases. In total these three categories caused 22 (or 59%) strong-evidence food-borne outbreaks, 210 (or 40%) human cases, 125 (69%) hospitalisations and 26 (or 70%) deaths. Food of non-animal origin caused two outbreaks and 34 cases.

The place of exposure (i.e. the location where the food was consumed or where the final stages of preparation of the food vehicle took place) was reported for 33 out of the 37 outbreaks, with nine at the household level, eight at the hospital or medical care facility, six as disseminated cases, three at a mobile retailer or market/street vendor and the remaining seven at another place of exposure. Thus, a substantial number of outbreaks occur in hospitals and other places of exposure where the proportion of individuals being vulnerable to infection with *L. monocytogenes* is higher than in the remaining population (Table 6) (Silk et al., 2014). The place of origin of the problem (i.e. the place where the contributory factors occurred) was reported for 24 outbreaks with 14 at the processing plant, two at the hospital or medical care facility and two at a restaurant, café, pub, bar, hotel or catering service. The number of outbreaks by year is as follows: 1 (2008), 4 (2009), 4 (2010), 4 (2011), 4 (2012), 8 (2013), 7 (2014), and 5 (2015).

Not all of these outbreaks are characterised by severe systemic forms of listeriosis. In 2015, Germany reported the largest *L. monocytogenes* (serovar 4b) outbreak affecting 159 cases, of which only two were hospitalised. This outbreak was associated with the consumption of mixed food (rice pudding) and occurred in a school or kindergarten (EFSA and ECDC, 2016). Without this outbreak, the three above-mentioned categories caused 61% of the strong-evidence food-borne outbreaks, 57% of the human cases, 69% of the hospitalisations and 70% of the deaths.

Most outbreaks successfully investigated in the EU-28 in recent years concerned animal-derived food or food composed partly from an animal-derived source (Table 6 and Appendix E). Mainly in the USA, large outbreaks of listeriosis have been reported in recent years where food commodities initially not considered as primary high-risk foods (Garner and Kathariou, 2016) have been implicated. These commodities included mainly produce (lettuce and fruit) and it should be noted that the first world-wide report on an outbreak of listeriosis in 1983 also occurred upon consumption of a plant food: coleslaw. Produce is assigned to the category of low-processed food commodities that may have a higher risk of pathogen transmission due to the rather simple processing chain applied. A cluster of more than 100 infections (147 cases) was reported in the USA in 2011 (McCollum et al., 2013) where epidemiological investigations confirmed that cantaloupe produced by a farm in Colorado was the outbreak source. Unsanitary conditions identified in the processing facility operated by the farm probably resulted in contamination of cantaloupes with *L. monocytogenes*. Another outbreak lasting from December 2014 to January 2015 caused 35 cases due to consumption of caramel apples.[32] This outbreak is of particular interest for modelling approaches: an outbreak occurred despite both the apple (pH < 4.0) and the caramel coating (free water activity < 0.8) having a physico-chemical profile that would deny a growth of *L. monocytogenes*. The hypothesis is that insertion of the stick led to a juicy interface between the apple and the coating. This growth-friendly microenvironment led to the observation that even under storage conditions of 7°C, *L. monocytogenes* could substantially grow (Glass et al., 2015). An outbreak of listeriosis linked to ice cream based milkshakes was associated with the exposure of a large number of consumers. The ice cream in this outbreak was also distributed to hospitals and severe illness was observed in four highly susceptible individuals. The exposure with high doses of *L. monocytogenes* was very unlikely. This outbreak suggests that human listeriosis cases could even occur after distribution of low-level contaminated products that do not support the growth of this pathogen if a highly vulnerable segment of the population is involved (Pouillot et al., 2015). Other foods of non-animal origin recently involved in listeriosis outbreaks were diced celery (in 2010 with 10 cases involved) (Gaul et al., 2013), frozen vegetables (in 2013–2016 with 9 cases involved[33]) and salads (in 2015–2016 with 19 cases involved[34]).

[32] http://www.cdc.gov/listeria/outbreaks/caramel-apples-12-14/index.html
[33] http://www.cdc.gov/listeria/outbreaks/frozen-vegetables-05-16/epi.html
[34] http://www.cdc.gov/listeria/outbreaks/bagged-salads-01-16/index.html

1539 **Table 6:** Summary of reported strong-evidence food-borne outbreaks in the EU/EEA as reported in the zoonoses database on occurrence of strong-
1540 evidence food-borne outbreaks caused by *Listeria* (2008–2015)

Food vehicle	Serovar[(m)] (number of outbreaks)	Number of outbreaks	Place of exposure[(n)] (number of outbreaks)	Place of origin[(o)] (number of outbreaks)	Human cases	Hospitalised cases[(p)]	Deaths[(p)]	Number of reporting countries	Distribution of outbreaks per country (year of outbreaks)[(q)]
Dairy		**4**			**44**	**42**	**11**		
Cheese[(a)]	1/2a (3), 1/2b (1)	4	D (1), H (3)	P (1), RT (1), U(2)	44	42	11	3	DE (2009), AT (2009), BE (2011, 2013)
Fish and seafood		**7**			**40**	**25**	**4**		
Crustaceans, shellfish, molluscs and products thereof[(b)]		3	H (1), M (2)	P (2), U(1)	10	8	2	2	UK (2013, 2013), FR (2013)
Fish and fish products[(c)]		4	D (1), H (1), O (1), U(1)	P (1), U(3)	30	17	2	3	DE (2010), DK (2010, 2014), NO (2013)
Meat and meat products		**11**			**126**	**58**	**11**		
Bovine meat and products thereof[(d)]	1/2a (1)	2	M (1), U(1)	M (1), P (1)	12	12	2	2	DK (2009), UK (2012)
Meat and meat products[(e)]		1	D (1)	U(1)	34	NR	NR	1	SE (2013)
Other or mixed red meat and products thereof[(f)]	1/2a (2)	3	D (2), HM (1)	P (2), U(1)	34	30	5[o]	3	UK (2010), FI (2012), SE (2014)
Pigmeat and products thereof[(g)]	1/2a (4), 4b (1)	5	H (2), R (1), Mu (1), O (1)	F (1), R (1), O (1), P (2)	46	28	6	5	AT (2008), CZ (2009), CH (2011), BE (2013), IT (2015)
Food of non-animal origin		**2**			**34**	**3**	**5**		
Vegetables and juices and other products thereof[(h)]	4b (2)	2	H (1), HM (1)	P (1), U(1)	34	3	5	2	DE (2013), CH (2014)
Other		**13**							
Bakery products[(i)]		2	H (1), D (1)	P (2)	16	16	1	2	FI (2011), UK (2012)
Buffet meals[(j)]		2	HM (1), R (1)	HM (1), R (1)	28	5	0	2	UK (2014), FI (2015)

Food vehicle	Serovar[m] (number of outbreaks)	Number of outbreaks	Place of exposure[n] (number of outbreaks)	Place of origin[o] (number of outbreaks)	Human cases	Hospitalised cases[p]	Deaths[p]	Number of reporting countries	Distribution of outbreaks per country (year of outbreaks)[q]
Mixed foods[k]	1/2 a (1), 4b (3), O4 (1)	7	HM (4), S (1), U(2)	C (1), HM (1), P (1), S (1), U(3)	192	17	2	5	UK (2011, 2012), DE (2014, 2015), DK (2014), PT (2015), SE (2015)
Other foods[l]		2	HM (1), O (1)	P (1), U (1)	45	4	1	2	UK (2010), DK (2014)
All		**37**	**D (5), H (9), HM (8), M (3), Mu (1), R (2), O (3), S (1), U (4)**	**C (1), F (1), HM (2), M (1), P (14), R (2), RT (1), O (1), S (1), U (13)**	**525**	**182**	**37**		

Note: More details can be found in Appendix E.

(a): Local produced soft cheese (*L. monocytogenes*, serovar unspecified), cheese (acid curd) made from pasteurised milk (*L. monocytogenes* serovar 1/2a), acid curd cheese (*L. monocytogenes* serovar 1/2a), more information about food vehicle not reported (*L. monocytogenes* serovar 1/2a), more information about food vehicle not reported (*L. monocytogenes* serovar 1/2b).

(b): Crab meat (*L. monocytogenes*, serovar unspecified), crab meat (*L. monocytogenes*, serovar unspecified), more information about food vehicle not reported (*L. monocytogenes*, serovar unspecified).

(c): Herring casserole in vegetable oil (*L. monocytogenes* serovar 4b), gravad salmon (*L. monocytogenes*, serovar unspecified), half-fermented trout (*L. monocytogenes*, serovar unspecified), smoked trout and smoked halibut (*Listeria* spp., unspecified).

(d): Beef stew (sous vide) (*L. monocytogenes*, serovar unspecified), pressed beef also called potted beef and beef stew (*L. monocytogenes* serovar 1/2a).

(e): More information about food vehicle not reported (*L. monocytogenes*, serovar unspecified).

(f): Tongue, beef, pork, ham, chicken, turkey (*L. monocytogenes* serovar 1/2a), meat jelly (*L. monocytogenes*, serovar unspecified), sausage (*L. monocytogenes* serovar 1/2a).

(g): Sliced jelly pork (*L. monocytogenes* serovar 4b), more information about food vehicle not reported (*L. monocytogenes* serovar 1/2a), more information about food vehicle not reported (*L. monocytogenes* serovar 1/2a), more information about food vehicle not reported (*L. monocytogenes* serovar 1/2a).

(h): Mixed salad (*L. monocytogenes* serovar 4b), pre-cut salad (*L. monocytogenes* serovar 4b).

(i): Sponge cake (*L. monocytogenes*, serovar unspecified), pork pies (*L. monocytogenes* serovar 4b).

(j): Sandwiches (*L. monocytogenes*, serovar unspecified).

(k): Sandwiches various and prepared salad dishes (*L. monocytogenes* O4), sandwiches (*L. monocytogenes*, serovar unspecified), iceberg lettuce with yogurt dressing, Gouda cheese (*L. monocytogenes* serovar 1/2a), composite meal (*Listeria* spp., unspecified).

(l): Salmon and cress sandwiches, egg mayonnaise sandwiches (*L. monocytogenes* O4), cold cuts (*Listeria* spp., unspecified).

(m): Serovar included when reported.

(n): Place of exposure: this is the location ('setting') where the food was consumed or where the final stages of preparation of the food vehicle took place. D = disseminated cases, HM = hospital or medical care facility, H = household, M = mobile retailer or market/street vendor, Mu = multiple places of exposure in one country, R = restaurant or cafe or pub or bar or hotel or catering service, O = others, S = school or kindergarten, U = unknown or not reported.

(o): Place of origin of the problem: place where the contributory factors occurred. C = canteen or workplace catering, F = farm, HM = hospital or medical care facility, M = mobile retailer or market/street vendor, P = processing plant, R = restaurant or cafe or pub or bar or hotel or catering service, RT = retail, O = others, S = school or kindergarten, U = unknown or not reported.

(p): The figure could be higher as for some outbreaks this was not reported.

(q): Austria (AT), Belgium (BE), Czech Republic (CZ), Denmark (DK), Finland (FI), France (FR), Germany (DE), Norway (NO), Sweden (SE), Switzerland (CH), United Kingdom (UK). Data from Spain has not been included in this table because it was provided outside the EFSA zoonoses database and in a different format of aggregation.

3.1.5. Epidemiological relationship between *L. monocytogenes* isolates of human and food origin along the food chain

Outbreaks of listeriosis occur around the globe every year and investigations have found associations with various food commodities. However, it is important to note that most human listeriosis cases are sporadic and until now sporadic cases of listeriosis were rarely traced to a food source due to methodological limitations. Due to the improved performance of epidemiological tools, case clusters are more effectively identified and the source can be more accurately traced. Next generation sequencing (NGS) has improved the detectability of outbreaks dramatically due to the fact that NGS can be applied on high numbers of isolates in a semi-automated way. Processing of raw sequence data nowadays is fully automated and harmonisation of data is simpler than with pulsed field gel electrophoresis or other molecular technologies. This advancement was also illustrated and confirmed by the outsourcing activity 3 when Møller Nielsen et al. (2017) studied the possible epidemiological relationship of 1,143 *L. monocytogenes* isolates collected in the EU, of which 333 were human clinical isolates and 810 were food-borne isolates.

Based on single nucleotide polymorphism (SNP), a total of 151 clusters were detected, including 124 novel clusters that had not previously been detected. Of these, 48 included one or more isolates from sporadic human cases, four clusters contained isolates from both sporadic cases and known outbreaks and 21 contained isolates from sporadic cases and food. This outsourcing activity illustrates the discriminatory power of WGS, demonstrating its ability to completely change the paradigm of outbreak investigation. WGS comparisons based on SNPs or cgMLST result in the detection of specific and sensitive potential links between human cases and/or foods that merit further epidemiological investigation.

Another objective of the outsourcing activity 3 (Møller Nielsen et al., 2017) was to apply a source attribution approach, and to partition the human disease burden to single sources. Five source attribution models were applied and five and four categories of sources (fish, swine, ovine, bovine and/or poultry) were considered. Given the small number of isolates, all of the isolates along the food chain that originate from a particular reservoir were combined. The capability to predict the correct source of strains in the dataset was evaluated. The source attribution models performed better than random. Depending on the number of loci used for attribution and based on self-attribution the best model predicted over 80% of strains to the correct source, while others predicted around 40% of sources correctly. The bovine source was found to be the main source for human disease in all of the models. Limitations of this study, as for source attribution in general, are the available set of strains and the corresponding information for classification and description of the strains. Especially in relation to *L. monocytogenes* it is extra cumbersome to attribute a strain to a specific food or animal source since contamination during processing is so important.

3.1.6. Analysis of Rapid Alert System for Food and Feed (RASFF) data on *L. monocytogenes*

RASFF data are used to qualitatively indicate the types and ranges of foods where *L. monocytogenes* has been recovered during the time period. In the RASFF database, under the product category 'food' and hazard '*Listeria monocytogenes*,' there were 760 notifications since 2008. The notifications were screened in duplicate and the majority, 91% (690/760) of notifications, were considered to be RTE foods. The number of notifications by RASFF product category and year can be found in Table 7.

The RTE foods included in the following three RASFF food product categories were most commonly notified: 'fish and fish products' (N = 288), 'milk and milk products' (N = 186) and 'meat and meat products other than poultry' (N = 126). A comparison of the RTE food RASFF notifications with strong-evidence food-borne outbreaks described in Section 3.1.4 indicates that food types associated with food-borne outbreaks are similar to the food types being controlled and found positive. The food categories associated with 59% of strong-evidence food-borne outbreaks were associated with 87% of RASFF notifications during this period. In particular, the 'dairy' food category was associated with 11% of the total outbreaks while the RASFF category 'milk and milk products' was related to 27% of the total RASFF notifications since 2008. Similarly, 'fish and seafood' and the RASFF category 'fish and fish products' were associated with 19% and 42% of outbreaks and notifications, respectively, while 'meat and meat products' were associated with 30% of the total outbreaks and the RASFF category

'meat and meat products other than poultry' with 18% of notifications. These findings reinforce that these food categories continue to have public health significance from a food safety perspective.

Table 7: Number of Rapid Alert System for Food and Feed notifications for *Listeria monocytogenes* by product category and year of notification and considered as ready-to-eat

Product category	Year									
	2008	2009	2010	2011	2012	2013	2014	2015	2016	2008–2016 period (percentage)
Fish and fish products	11	26	39	54	22	27	43	35	31	288 (41.7)
Meat and meat products (other than poultry)	10	10	15	17	17	12	13	16	16	126 (18.3)
Milk and milk products	22	13	15	23	20	20	29	30	14	186 (27.0)
Cereals and bakery products		1								1 (0.1)
Cocoa and cocoa preparations, coffee and tea		1								1 (0.1)
Crustaceans and products thereof		3	4	1	4	1		1	2	16 (2.3)
Eggs and egg products	1									1 (0.1)
Fats and oils									1	1 (0.1)
Fruit and vegetables	1			2	5	1	5	4		18 (2.6)
Gastropods			1							1 (0.1)
Herbs and spices									1	1 (0.1)
Ices and desserts					1					1 (0.1)
Nuts, nut products and seeds							1	1		2 (0.3)
Other food product / mixed		1			2			2		5 (0.7)
Poultry meat and poultry meat products	1	2	2	1	1	3	1	2	4	17 (2.5)
Prepared dishes and snacks		1	4	1	2	5	2	2	7	24 (3.5)
Soups, broths, sauces and condiments								1		1 (0.1)
All product categories	46	58	80	99	74	69	94	94	76	690

3.1.7. Summarising remarks for hazard identification

- The reported number of confirmed human listeriosis cases in the EU/EEA was 60% higher in 2015 (2,206 cases) than in 2008 (1,381 cases). Under-reporting/under-ascertainment of listeriosis cases has been estimated at around a factor of 2 in the UK and North America.
- Most listeriosis cases are sporadic and almost all (> 98%) human *L. monocytogenes* infections are acquired domestically and most travel-related cases have acquired the infection within the EU/EEA.
- The highest notification rates (i.e. notified incidence rates) of listeriosis in the EU/EEA are commonly seen in the elderly, over 65 years old, and in children under 1 year of age. In 2015, the rate for males was double that for females in the age group over 65 years. In the same year, the notification rate for males was over 140 times higher than for males in the age group 5–14 years, while the respective rate ratio was 24 for females.
- In the age group 15–24 years, the reported proportion of pregnancy-associated cases increased from 50% in 2013 to 75% in 2015, whereas no marked change was seen in the female age group 25–44 years.
- In 2015, 270 deaths were reported in the EU/EEA, which was the highest annual number of deaths reported due to listeriosis since 2008. The overall CFR was 17.7% in 2015. For those

over 45 years old, the average annual CFR in the period 2008–2015 ranged in females from 16.1% among 45–64-year-olds to 22.5% for those over 75 years old, and in males from 16.0% for the 65–74-year-olds to 20.3% for those over 75 years old.

- There were no significant differences in CFRs between genders across age groups except for the age group 21–44 where the case fatality was almost four times higher for males than for females.
- In addition to old age and increased susceptibility due to underlying conditions, medical practices and medications have been hypothesised as risk factors for listeriosis. The use of proton pump inhibitors (PPI), which increase gastric pH, was associated with increased susceptibility to non-pregnancy-related listeriosis in Denmark with an adjusted OR of 2.81 (95% CI, 2.14–3.69). Based on the adjusted OR the population-attributable fraction of listeriosis due to current PPI usage was estimated at 8.3%.
- In RASFF, 91% of the 760 notifications since 2008 related to *L. monocytogenes* were considered to be RTE food. The following three RASFF food product categories were most commonly notified: 'fish and fish products' (N = 282), 'milk and milk products' (N = 186) and 'meat and meat products other than poultry' (N = 112). Together these RASFF categories accounted for 87% of the 690 notifications.
- Comparisons between RASFF notifications and strong-evidence listeriosis food-borne outbreaks indicate that food types being controlled and found positive are often similar to the food types associated with outbreaks. This finding reinforces the fact that these food categories continue to have public health significance from a food safety perspective.
- A total of 37 strong-evidence food-borne outbreaks caused by *Listeria* were reported in the EU/EEA with 525 human cases (i.e. less than 4% of total reported cases during the period), 182 hospitalisations and 37 deaths during 2008–2015. The 'meat and meat products' food category was responsible for 11 of these outbreaks, causing 126 cases. 'Fish and seafood' and 'dairy' food categories were responsible for respectively seven and four of these outbreaks, causing 40 and 44 cases. In total these three categories caused 59% of the strong-evidence food-borne outbreaks and 40% of the human cases.
- Recent outbreak reports such as those associated with cantaloupe and caramel apples in the USA demonstrate that as yet unconsidered RTE food categories of plant-derived origin under certain conditions can also support growth and have the potential to contribute to the burden of disease.
- Considering the place of exposure when reported, 28% of the outbreaks were reported at the household level, 25% at a hospital or medical care facility, 16% as disseminated cases, 9% at a mobile retailer or market/street vendor and another 22% at another place of exposure.
- The discriminatory power of genotyping by sequencing for outbreak detection was suggested through the outsourcing activity 3. They detected 124 previously not described clusters of strains. Of these, 48 included one or more sporadic human isolates of which four clusters contained both sporadic cases and known outbreaks and 21 contained both sporadic cases and food. Thus seemingly unrelated sporadic cases of listeriosis could be traced back to food sources. However, epidemiological information is still needed to further investigate the microbiological clusters.
- A source attribution study through outsourcing activity 3 on 333 human clinical and 810 food-borne isolates collected in the EU, indicated that the highest share of the human disease burden is attributed to the bovine source using a number of different models. The source attribution models performed better than random and the best model based on self-attribution of strains predicted over 80% of strains to the correct sources. Limitations of this study, as for source attribution in general, are the available set of strains and the corresponding information for classification and description of the strains. Especially in relation to *L. monocytogenes* it is extra cumbersome to attribute an isolate to a specific food or animal source since contamination during processing is so important. These limitations make the conclusions on source attribution uncertain.

3.2. Evidence for hazard characterisation

3.2.1. Biology and virulence of *L. monocytogenes*

Clinical biology of *L. monocytogenes*

Listeria monocytogenes has been isolated from more than 40 mammalian and avian species and both humans and animals develop similar forms of disease. After ingestion and passage through the stomach, *L. monocytogenes* multiplies in the intestinal lumen, crosses the intestinal barrier, enters the bloodstream and accumulates in the liver and spleen. Thereafter, the bacteria can re-enter the bloodstream to cause central nervous infection or abortion (Vazquez-Boland et al., 2001). In healthy individuals, infection with *L. monocytogenes* may cause a gastroenteritis syndrome. Outbreak reports have shown that even a very high contamination level of the food source might only lead to this milder form of listeriosis (Dalton et al., 1997). Moreover, *L. monocytogenes* can be isolated in some cases from rare sites of infection in the human body such as ankles, eyes and kidneys.

Strains of *L. monocytogenes* can be grouped into four evolutionary lineages (I-IV), and 13 serotypes. However, strains of only three serotypes (1/2a, lineage II; 1/2b and 4b, lineage I) have been associated with 98% of all human listeriosis cases (Orsi et al., 2011). Lineage I encompasses the clinically relevant serovars 1/2b and 4b whereas serovar 1/2a is accounted to lineage II (Lomonaco et al., 2015).

Organisation of important molecular traits for *Listeria* virulence

With the availability of the first full genome of *L. monocytogenes* EGD in 2001, most experts expected a rapid growth in the number of biomarkers that indicate distinct *Listeria* pathotypes (Glaser et al., 2001). This, however, turned out not to be the case. In the meanwhile, multiple studies have shown that the core genome of *L. monocytogenes* is a stable feature. Most genetic rearrangement is conferred through uptake of mobile elements such as plasmids and transposons. Transposons integrate at preferred sites (hotspots) into the *L. monocytogenes* genome and some of these hotspots are hot candidates in terms of an improved understanding of adaptation against environmental stresses. The main findings in the whole genome sequencing study by Møller Nielsen et al. (2017) are in line with the 'stable core genome theory.' Although a huge number of virulence associated genes (N = 115) were tested, more than 80% of the putative marker genes were detected in more than 95% of the test strains of lineage I and II. This finding proves that most virulence markers are ubiquitous to the most important genetic lineages. The majority of markers that were not present in the majority of strains were found in food-borne strains, of those mostly representatives of lineage II.

Four pathogenicity islands have been described in *L. monocytogenes* and *L. ivanovii*: LIPI-1 contains a couple of the major virulence factors such as *hly* (encodes for a hemolysin), *plcB* (encodes for phospholipases needed for *L. monocytogenes* release into the cytosol), *actA* (encodes the listerial surface protein ActA required for Actin-based intracytoplasmic movement and cell-to-cell spread) and is present in all lineages. Some other important virulence factors mediating entry into host cells such as internalin A and B are encoded by an *inlAB* operon located outside the classical LIPI-1. LIPI-2 contains a sphingomyelinase specific to *L. ivanovii* and additional internalin genes and was described in *L. ivanovii* (Vazquez-Boland et al., 2001; Dominguez-Bernal et al., 2006). LIPI-3 encodes for an additional hemolysin called streptolysin S and was most frequently found in clinically relevant lineage I *L. monocytogenes* strains (Molloy et al., 2011). This finding was confirmed by the whole genome sequencing study by Møller Nielsen et al. (2017), who showed that the LIPI-3 genes and the gene for the virulence protein Vip (*vip* gene (Cabanes et al., 2005)) were more likely present in clinical and/or lineage I isolates. A fourth pathogenicity island has been very recently described and contains six genes encoding for a cellobiose-family phosphotransferase system (Maury et al., 2016).

Many of the more than 80 virulence factors known in *L. monocytogenes* are regulated by the transcriptional regulator PrfA (Freitag et al., 2009). A number of surface proteins including the internalins are crucial for host cell invasion (Bierne et al., 2007). Internalin A (InlA), which interacts with E-cadherin present at the surface of the host cell, mediates the entry of *L. monocytogenes* into intestinal epithelial cells (Bonazzi et al., 2009). Several mutations in the *inlA* gene lead to a premature stop codon and subsequently in a truncated InlA protein. An overview of gene mutations in *L. monocytogenes* leading to a reduced virulence is provided in Appendix F. These types of mutations,

which are carried presumably by environmental and food-borne strains, are associated with attenuated virulence and often found in food-borne isolates (Nightingale et al., 2008; Van Stelten et al., 2010). After *L. monocytogenes* enters the host cell, it escapes the vacuole, replicates intracellularly and spreads from cell to cell (Cossart, 2011). These processes are mainly mediated by the pore-forming toxin listeriolysin O, encoded by the *hly* gene, and products from the *plc*B gene and other virulence factors (Gedde et al., 2000; Hamon et al., 2012). Some researchers have undertaken further attempts to unravel virulence genes that are associated with higher frequency in either lineage I/II or III/IV. Interesting results suggested carbon source utilisation and tolerance of bile stress as possible triggers for a different pathogenic potential (reviewed in Lomonaco et al. (2015)). As mentioned before, the ability to sequence a vast number of isolates was a leap forward in recent years but proved that the core genome of *L. monocytogenes*, including most virulence-associated genes, is rather stable and that most adaptation occurs through mobile elements at a limited number of genetic hotspots (Kuenne et al., 2013). A limitation of sequencing is that gene mapping generates hypotheses but lacks information on whether post-genetic events could render the proposed effects. A solution to this problem is the use of cell culture for virulence models and animal challenges to study *L. monocytogenes* pathogenicity *in vivo*. Approaches differ widely and it is remarkable that *in vivo* data cannot be deduced from *in vitro* cell culture-based data (Disson and Lecuit, 2013). Intravenous, subcutaneous or intraperitoneal infection of rodents were the most frequently used animal studies; however, all these methods of administering strains do not mimic the natural route of exposure. Oral infections of mice would follow the natural route of exposure but were shown to be biased due to a genetic difference between murine and human E-cadherin in epithelial cells (Lecuit et al., 1999). Transgenic mice (hEcad) have overcome this problem to some extent but are not commercially available (Lecuit and Cossart, 2001). The only other animal model leading to a course of infection comparable to the infection in humans after oral exposure is the guinea pig model. Other model organisms for virulence studies such as gerbils and wax moths are not easily manageable or far from representative of the situation in humans.

Virulence variability in *L. monocytogenes*

Through the aforementioned studies, it has become clear that *L. monocytogenes* demonstrates enormous serotype/strain variation in virulence and pathogenicity levels. Epidemic strains from foods are highly infective and sometimes deadly while food or food environment isolates are less associated with human cases and are less virulent mainly due to mutation in the main virulence genes (reviewed by Velge and Roche (2010)). Some listeriosis outbreaks were traced back to foods carrying more than one *L. monocytogenes* strain of different serotypes and virulence profiles (Gilmour et al., 2010; Laksanalamai et al., 2012; Rychli et al., 2014). Until recently there was no comprehensive definition of virulence levels of *L. monocytogenes* that could address the risk assessment aspects of either hypervirulence or hypovirulence (Velge and Roche, 2010). A milestone was reached with a study that compared epidemiological results based on genetic typing with sequence information and results from animal models. The study from France tested more than 6,000 isolates from both clinical specimens and food items and showed that almost 80% of isolates could be assigned to only 12 clonal complexes (CCs). The clones that were more frequently isolated from clinical samples, `infection-associated´, were different from the clones more frequently isolated from food samples, `food-associated´. There were also clones that were `intermediate´. Clones CC1, CC2, CC4 and CC6 were to a high probability of clinical origin, whereas CC121 and CC9 were strongly associated with provenance from food. The `infection-associated´ CCs were most commonly associated with central nervous system (CNS) and maternal–neonatal (MN) infections as opposed to isolated bacteraemia. The `food-associated´ CC121 and CC9 were rarely present in clinical samples but, if recovered from clinical specimens, usually isolated from blood (Maury et al., 2016). The latter CCs were also more frequently associated with highly immunocompromised patients or patients showing a higher number of severe comorbidities. Using a humanised mouse model it became evident that the food-associated CCs were less invasive and therefore of a hypovirulent state. Strain sequencing at least partly strengthened the argument that clonal complexes encompassing hypovirulent strains are more likely show mutations in the internalin A gene, which had already been proven for MLST 121 strains in a previous study (Schmitz-Esser et al., 2015; Maury et al., 2016).

The outcomes of this study were only partly confirmed by the results of the study by Møller Nielsen et al. (2017). There, a lower number of strains (1,143) were sequenced, isolated during the BLS or mainly during a period of two years in different laboratories from different clinical or food-borne

sources. In this study, the isolates of CC121 and CC9 were predominantly food-borne, which supports the study from Maury et al. (2016). A clear assignment of isolates of CC1, CC2, CC4 and CC6 to a clinical origin was only substantiated for isolates of CC1 and CC4 (see Figure 8). A detailed analysis of isolates of food-borne origin only revealed that strains of CC121, CC8, and CC155 were predominately isolated from fish and fish products whereas strains of CC31 and CC2 showed higher frequency in meat and meat products. Since the sequenced isolate collection was arbitrarily put together, mainly incorporating isolates from the BLS and a limited sampling interval of two years, conclusions from these results should be taken with care.

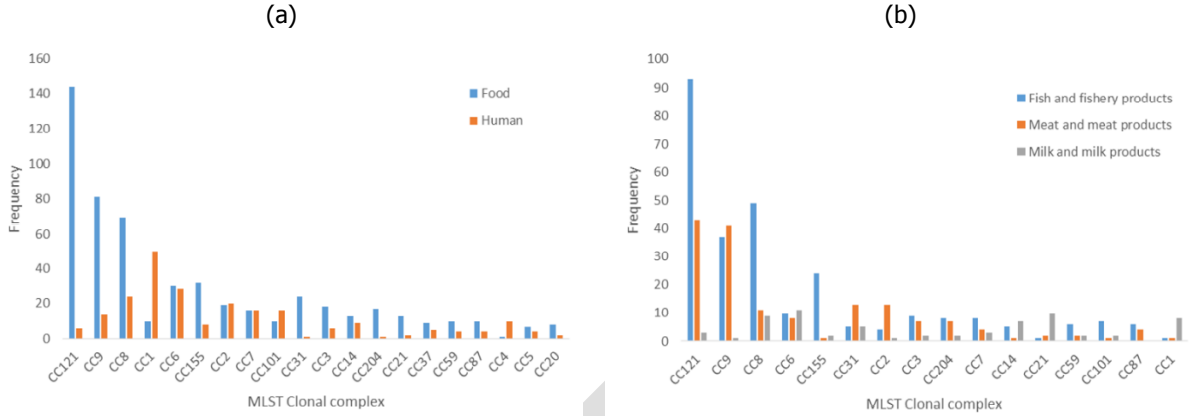


Figure 8: Distribution of clonal complexes (CCs) as assigned by whole genome sequencing in ready-to-eat foods and from sporadic human clinical infections (a) and from the three major food product categories (b)

Environmental and host-related factors impacting on virulence

The elucidation of the infection pathway of *L. monocytogenes* in recent decades has shown that virulence is not a stable characteristic but can be influenced by environmental conditions. So far only a limited number of studies (reviewed by NicAogain and O'Byrne (2016)) investigated the impact of food on the *in vitro* and *in vivo* virulence of *L. monocytogenes*. Temperature, osmotic stress and pH were shown to have an impact on the virulence profile (Andersen et al., 2007; Duodu et al., 2010; Walecka et al., 2011). Milk and milk-specific characteristics, like fat content, were demonstrated to have an influence on the *in vitro* virulence of *L. monocytogenes* as well (Pricope-Ciolacu et al., 2013). Conclusively, Mahoney and Henriksson (2003) reported that the pathogenicity of *L. monocytogenes* depends on the nature of the food in which the pathogen is present and Rantsiou et al. (2012) determined that food matrices alter strain-dependently the expression of several major virulence factors. A recently published study encompassing phenotypic and sequencing approaches found that stress tolerance of *L. monocytogenes* is associated with serotype, CC, full length *inl*A gene profiles, and the presence of plasmids. Interestingly, isolates with full length *inl*A exhibited enhanced cold tolerance relative to those harbouring a premature stop codon in this gene (Hingston et al., 2017). The limitation of all these studies is that they focus on experimental environments or food as only one step of a sequence of events during natural *L. monocytogenes* infection. *Listeria monocytogenes* needs to survive not only in the food environment but also under conditions encountered during the passage through the gastrointestinal tract of the host and to be subsequently able to cross the intestinal, placental and blood–brain barriers (Lecuit, 2005). All these steps affect the virulence of the pathogen and point towards a finely tuned process that enables *L. monocytogenes* to infect hosts. When exposed to adverse conditions, like the food environment or the gastrointestinal tract, *L. monocytogenes* shapes its transcriptome by activating complex response networks related not only to stress but also to virulence. The main stress response regulator, the alternative sigma factor σ^B, contributes directly to the regulation of virulence gene expression like *inlA*, *inlB* and *prfA* under conditions typically encountered during gastrointestinal passage (Nadon et al., 2002; Kazmierczak et al., 2003; Sue et al., 2004). The pathogenicity of *L. monocytogenes* has been related to the viability of the pathogen in the acidic environment of the stomach and subsequently in the presence of bile in the small intestine (Jiang et al., 2010). *L. monocytogenes* gene *bsh*, positively regulated by PrfA, encodes for a bile salt hydrolase that contributes to survival in the gastrointestinal tract and is involved in the

intestinal and hepatic phases of listeriosis (Dussurget et al., 2002; Begley et al., 2005). However, the specific effects on pathogenicity and subsequently on health risk are still not completely understood.

Virulence heterogeneity and detection of *L. monocytogenes*

A non-trivial point for interpreting the concepts of clinical and food-related strains is the impact of hypo/hypervirulence on *L. monocytogenes* detectability in different matrices. Initial studies published in 2003 have shown that hypovirulent strains appear less frequently on some isolation media (Gracieux et al., 2003). A follow-up study demonstrated that the effect was most likely due to the composition of the detection media (e.g. antimicrobials added) rather than due to mutations in virulence regulator genes such as *prf*A (Roche et al., 2009). The detection of *L. monocytogenes* from food during selective enrichment can also be limited by the natural microbiota or by other *Listeria* spp. (Cornu et al., 2002; Zitz et al., 2011; Keys et al., 2013; Dailey et al., 2014; Dailey et al., 2015). It was furthermore suggested recently that strain competition within the species *L. monocytogenes* is one of the factors related to bias during the enrichment and detection procedure in the case of mixed cultures, as a consequence of strain fitness in a given niche like food or other enrichment conditions (Gorski et al., 2006; Zilelidou et al., 2016b). In the case of a food product contaminated with multiple *L. monocytogenes* strains, the strain with the growth disadvantage will be missed during enrichment (Zilelidou et al., 2016a). The Jameson effect, or the growth inhibition due to a lack in nutrient availability, gives a competitive advantage to the numerically dominant species (Mellefont et al., 2008). Anyhow, this was not the case for *L. monocytogenes* co-cultures as growth competition also occurs between *L. monocytogenes* strains with similar growth rates (Zilelidou et al., 2016b). Inhibition of growth through production of bacteriocins or bacteriophages was also proposed (Cornu et al., 2002). One can speculate that the newly discovered recombination hotspot repeat genes in the genome of often food-associated ST121 strains, suggested to be involved in cell–cell interactions, might provide a better competition against other bacteria or other *L. monocytogenes* strains (Schmitz-Esser et al., 2015). However, future work is needed to confirm this hypothesis as a critical role of cell contact in growth inhibition and virulence competition has already been shown (Zilelidou et al., 2015). The advantage of certain *L. monocytogenes* strains during competition could not be correlated with the serotype (Gorski et al., 2006; Zilelidou et al., 2016b) even if a lineage-dependent detection of strains during enrichment (Bruhn et al., 2005) and a competitive advantage of serotype 1/2a strains over serotype 4b in biofilm formation were reported (Pan et al., 2009).

The presence of more than one *L. monocytogenes* isolate in food can lead to increased infection rates due to synergistic effects on the virulence potential. Specifically, in co-cultivation experiments *L. monocytogenes* isolates considered strong growth competitors, as their growth was not or only slightly attenuated by other isolates, showed high invasiveness compared to weak fitness competitors (Zilelidou et al., 2015). Furthermore, *L. monocytogenes* isolates classified as virulent reach significantly higher cell counts on selective agar media than non-virulent bugs in single cultures (Gracieux et al., 2003). It was speculated that cell contact co-cultivation of *L. monocytogenes* isolates can lead to an induction of virulence gene expression for strong competitor strains and might trigger strain competition for entry into the host cells (Zilelidou et al., 2015). However, further confirmation through gene expression studies is needed.

3.2.2. Clinical picture of reported human listeriosis cases in the EU/EEA

The population groups at highest risk for severe listeriosis are the elderly, pregnant women and those with underlying immunosuppressive conditions (Maertens De Noordhout et al., 2016; Pfaff and Tillett, 2016). Over 97% of human listeriosis cases reported in the EU/EEA with available data were hospitalised (EFSA and ECDC, 2016), reflecting the focus of EU-level surveillance on invasive forms of the disease. In a Belgian study by Bertrand et al. (2016), cancer was the most common (43%) underlying condition in human listeriosis cases for all serotypes with known data (N = 426) followed by digestive diseases with 12% (46% with no indication of underlying condition).

In the pooled TESSy data from 2011 to 2015,[35] information on clinical specimen type was reported for almost 3,600 cases (39.2% of all reported cases during that time period). Of these, 71.8% were

[35] TESSy data as of 20 December 2016.

septicaemia (specimen = blood) and 19.4% meningitis (specimen = cerebrospinal fluid, CSF), while 8.4% of the samples were from 'other sterile site' (these are not specified but could be, e.g. joints, heart or eyes). Only 11 positive samples (0.3%) were reported from a non-sterile site (e.g. placental tissue), all from females under 44 years old. Bloodstream infections were relatively more common (45.1%) in the very elderly (over 75 years old) whereas meningitis was mainly (29.6%) reported in the middle-aged group (45–64 years old) (Table 8).

Table 8: Specimen[(a)] types of reported invasive listeriosis cases by age groups in the EU/EEA, N = 3,597, 2011–2015

Age (years)	Blood	CSF	Other sterile site	Non-sterile site
	N (%)	N (%)	N (%)	N (%)
< 1	71 (2.7)	16 (2.3)	9 (3.0)	3 (27.3)
1–20	37 (1.4)	29 (4.1)	9 (3.0)	1 (9.1)
21–44	249 (9.7)	92 (13.1)	90 (29.6)	7 (63.6)
45–64	515 (20.0)	205 (29.3)	69 (22.7)	0 (0.0)
65–74	545 (21.1)	177 (25.3)	53 (17.4)	0 (0.0)
≥ 75	1,165 (45.1)	181 (25.9)	74 (24.3)	0 (0.0)
Total	2,582 (100.0)	700 (100.0)	304 (100.0)	11 (100.0)

CSF: cerebrospinal fluid, N: No of cases with the specimen type used for diagnosis of case; %: Percentage of cases with a specimen type in an age group.
Source: Data from The European Surveillance System – TESSy, provided by Austria, Croatia, Estonia, France, Hungary, Lithuania, Luxembourg, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, United Kingdom and released by ECDC. Age was missing for one case with specimen type 'blood.'
(a): Specimen types were introduced to EU-level reporting in 2012.

Tables 9 and 10 show the CFR values in the different age groups and each gender. For infections caused by *L. monocytogenes* serogroup IIa, the CFR was significantly lower in the female age group 1–44 years with no significant differences between other age and gender groups (Table 9). A significantly lower CFR was also estimated in the female age group 1–44 years for infections caused by *L. monocytogenes* serogroup IVb. An association with CFR and age was noted for serotype IVb (Table 10). The results are further visualised in Figure 9. As the infections with serogroup IVb are most commonly reported in humans in the EU/EEA, the severity of these infections is noteworthy and requires further study.

Table 9: Case fatality rates in males and females in different age groups in invasive human infections with *Listeria monocytogenes* serogroup IIa, pooled data, 2007–2015

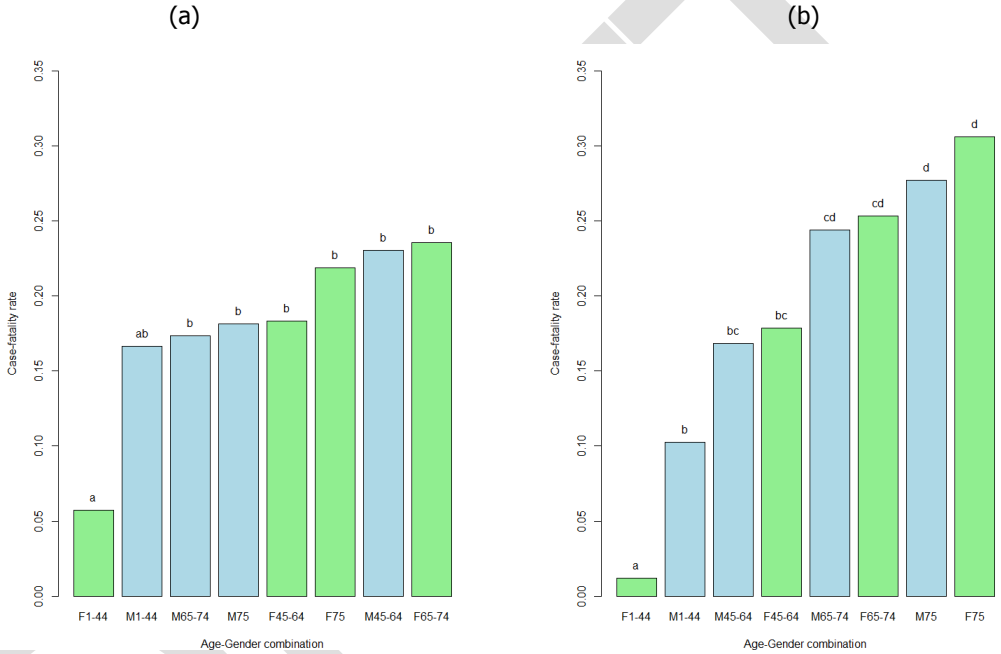
	Males (N = 779)				Females (N = 715)			
Age (years)	**Total**	**Death**	**CFR**	**Group[(a)]**	**Total**	**Death**	**CFR**	**Group[(a)]**
1–44	36	6	0.17	b	105	6	0.06	a
45–64	204	47	0.23	b	142	26	0.18	b
65–74	219	38	0.17	b	157	37	0.24	b
≥ 75	320	58	0.18	b	311	68	0.22	b

CFR: case fatality rate.
Source: Data from The European Surveillance System – TESSy, provided by Austria, Belgium, France, Germany, Hungary, Ireland, Italy, Lithuania, Luxembourg, Netherlands, Norway, Poland, Portugal, Romania, Slovenia, Sweden, United Kingdom and released by ECDC.
(a): Letters are used to indicate which CFR values are significantly different. For example, when CFR values are not significantly different they will have the same letter and values that are significantly different will have a different letter (i.e. a, a, b would mean that the first and second groups both differ from the third group but not between each other).

Table 10: Case fatality rates in males and females in different age groups in invasive human infections with *Listeria monocytogenes* serogroup IVb, pooled data, 2007–2015

Age (years)	Males (N = 1025)				Females (N = 789)			
	Total	Death	CFR	Group[(a)]	Total	Death	CFR	Group[(a)]
1–44	78	8	0.10	b	164	2	0.01	a
45–64	285	48	0.17	bc	151	27	0.18	bc
65–74	254	62	0.24	cd	154	39	0.25	cd
≥ 75	408	113	0.28	d	320	98	0.31	d

CFR: case fatality rate.
Source: Data from The European Surveillance System – TESSy, provided by Austria, Belgium, France, Germany, Hungary, Ireland, Italy, Lithuania, Luxembourg, Netherlands, Norway, Poland, Portugal, Romania, Slovenia, Sweden, United Kingdom and released by ECDC.
(a): Letters are used to indicate which CFR values are significantly different. For example, when CFR values are not significantly different they will have the same letter and values that are significantly different will have different letter (i.e. a, a, b would mean that the first and second groups both differ from the third group but not between each other').



F: female (green bar); M: male (blue bar).
Case fatality rate values not significantly different have the same letter (comparisons only within groups). Multiple comparison analysis conducted in each serogroup separately with alpha = 0.1.

Figure 9: Case fatality rates in different age–gender groups in invasive human infections with *Listeria monocytogenes* serogroup IIa (a) and serogroup IVb (b), pooled data, 2007–2015

3.2.3. *Listeria monocytogenes* dose–response relationships

The conceptual process upon which microbial DR models are developed comprises four biologically plausible steps: i) ingestion of an assumed number of organisms by a host individual; ii) ingested organisms passing through the various barriers and surviving until they reach the target site; iii) surviving organism(s) resulting in infection; and iv) infection resulting in illness. The current DR models are specified upon the assumptions that encompass all four probabilistic elements involved in this process: random number of ingested organisms, random number of surviving organisms and reaching the target site, concentration-independent probability of surviving organisms resulting in infection; and of illness following infection. Therefore, within the microbial DR models framework, it is generally accepted that the minimal infective dose is one cell associated with a probability of infection or illness (r). If each cell is capable of inducing illness ('single-hit'), then the probability of illness given a known number of ingested cells D can be derived from:

$$P_{ill}(D,r) = 1 - (1-r)^D \qquad \text{Equation 13}$$

The underlying assumption of the single-hit model is then the absence of interaction between the ingested cells where *r* is assumed to be independent of the size of the ingested dose. The interpretation of the probability derived from the single-hit model means that the single-hit model provides a conditional probability of illness given a value of *r* and a number of ingested bacteria which roughly represents the outcome of the interaction between the individual characteristics of the exposed host, the bacterial strain characteristics and the food characteristics. Thus, the parameter *r* is expected to be highly variable and should be specified for each single exposure occasion.

If the variability in the parameter *r* is addressed, with the function f(*r*) being the probability distribution for *r*, the probability of illness can be derived from:

$$P_{ill}(D) = \int_0^1 [1 - (1 - r)^D] f(r) dr \qquad \text{Equation 14}$$

This new probability of disease has a different interpretation from that calculated with the single-hit model. It represents the marginal probability of illness given an exposure to *D* organisms. By marginal we mean here the probability of illness in an exposed population (average probability of illness).

To capture the variability of the parameter *r*, different approaches are used: including a full characterisation of the probability distribution of *r* or a stratification of the exposed population. In the latter approach, different values of *r* for different segments of the population and no variability within each segment of the population are assumed. In doing so, only variability attributable to host group factors is integrated. The general approach to estimate *r* is by combining an extensive exposure assessment encompassing 'all' RTE foods with epidemiological data on the observed number of cases and relative risk of the different population segments (for an example, see Table 11). The *r* values are then optimised so that the estimated number of cases matches the observed number of cases.

Commonly, the original single-hit response model is replaced by the single-hit exponential DR model (named exponential model). In this model it is assumed that the actual ingested dose is uncertain but can be described by a Poisson distribution with a mean equal to λ:

$$P_{ill}(D, r) = 1 - \exp(-\lambda r) \qquad \text{Equation 15}$$

Exponential DR models have been extensively used for the characterisation of the *L. monocytogenes* DR relationship (Farber et al., 1996; Notermans et al., 1998; Lindqvist and Westoo, 2000; Chen et al., 2003; FDA and FSIS, 2003; Franz et al., 2010; Mataragas et al., 2010; Tromp et al., 2010; Busschaert et al., 2011; FAO and WHO, 2014; Sant'Ana et al., 2014; Vasquez et al., 2014).

Using epidemiological data, the Food and Agriculture Organization and the World Health Organization (FAO and WHO, 2004) estimated *r*, the probability of illness after consumption of one cell of *L. monocytogenes*, at around $r_1 = 5 \times 10^{-12}$ for susceptible host individuals (immunocompromised persons, pregnant women, and elderly persons), and $r_2 = 5 \times 10^{-14}$ for non-susceptible persons. Including uncertainty, the 5th percentiles are 2.47×10^{-13} for r_1 and 3.55×10^{-15} for r_2, and the 95th percentiles are 9.32×10^{-12} for r_1 and 2.70×10^{-13} for r_2.

In the Food and Drug Administration and Food Safety and Inspection Service risk assessment (FDA and FSIS, 2003), DR relationships for invasive listeriosis were characterised for three population groups: (i) the perinatal (fetuses and neonates infected *in utero* by contaminated foods consumed by their mothers), (ii) elderly people (60 years old and older), and (iii) the intermediate-age population (including healthy individuals and certain susceptible subpopulations, such as AIDS patients or individuals under immunosuppressive therapy). Five different DR models fitted to one dataset obtained from mice infected with a single strain of *L. monocytogenes* (Golnazarian et al., 1989) were used to characterise the model uncertainty, although the exponential model received the greatest weight as it was the best-fitting model. The variability in virulence of *L. monocytogenes* strains was estimated on the basis of mice experiments, and variability in host susceptibility was estimated based on mice studies and human epidemiological data. Furthermore, FoodNet surveillance data on the incidence rates of listeriosis in the USA were used to adjust the mortality curves to reduce the resulting overestimation of listeriosis risk, reflecting the different susceptibility between mice and humans (FDA and FSIS, 2003; Hoelzer et al., 2013).

The exponential DR model for *L. monocytogenes* has recently been applied, also taking into account the actual heterogeneity observed in the pathogen–host interaction by means of a probability distribution for the parameter *r* (Mataragas et al., 2010; Gkogka et al., 2013; Sant'Ana et al., 2014;

Pouillot et al., 2015). Pouillot et al. (2015) carried out a refinement of the exponential model used in the FAO and WHO *L. monocytogenes* risk assessment (FAO and WHO, 2014) to more adequately represent extremely susceptible population subgroups and highly virulent *L. monocytogenes* strains. A model incorporating adjustments for variability in *L. monocytogenes* strain virulence and host susceptibility was derived for 11 population subgroups with similar underlying comorbidities using data from multiple sources, including human surveillance and food survey data.

Table 11: Epidemiological data used to assess the dose–response model of Pouillot et al. (2014)

Subpopulation	Number of individuals in France (Goulet et al., 2012) (A)	Listeriosis cases in France (2001–2008) (B)	Proportion (C) = (A/total of A)	RR (D) = (B/A) × (Total of A/Total of B)	Expected confirmed cases in EU/EEA 2008–2015 (D x C x total cases in EU/EEA)	Percentage of expected confirmed cases in EU/EEA 2008–2015
Under 65 years old, no known underlying condition (i.e. 'healthy adult')	48,909,403	189	0.767	0.126	1,351	9.65
Over 65 years old, no known underlying condition	7,038,068	377	0.110	1.743	2,695	19.24
Pregnancy	774,000	347	0.012	14.591	2,480	17.71
Non-haematological cancer	2,065,000	437	0.032	6.887	3,123	22.31
Haematological cancer	160,000	231	0.003	46.988	1,651	11.79
Renal or liver failure (dialysis, cirrhosis)	284,000	164	0.004	18.794	1,172	8.37
Solid organ transplant	25,300	16	0.0004	20.582	114	0.82
Inflammatory diseases (rheumatoid arthritis, ulcerative colitis, giant cell arteritis, Crohn's disease)	300,674	68	0.005	7.361	486	3.47
HIV/AIDS	120,000	22	0.002	5.967	157	1.12
Diabetes (type I or type II)	2,681,000	79	0.042	0.959	565	4.03
Heart diseases	1,400,000	29	0.022	0.674	207	1.48
Total	63,757,445	1,959	1		14,002	

Note: Number of persons with underlying conditions and number of cases of invasive listeriosis observed in France, 2001–2008 used by Pouillot et al. (2015); and expected number of invasive human listeriosis cases per population segments in the EU/EEA (2008–2015) estimated based on the French data in this Scientific Opinion.
HIV/AIDS: Human immunodeficiency virus infection and acquired immune deficiency syndrome.

The lognormal-Poisson DR model was chosen and proved able to reconcile DR relationships developed based on surveillance data with outbreak data. In comparison, the classical beta-Poisson DR model was insufficiently flexible for modelling *L. monocytogenes* DR relationships, especially in outbreak situations. Overall, the modelling results suggest that most listeriosis cases are linked to the ingestion of food contaminated with medium to high ($3.5 - 7.5 \log_{10}$ CFU/serving) concentrations of *L. monocytogenes*. The relationship derived by Pouillot et al. (2015) can be considered an 'extended' exponential model, which encompasses the risk of listeriosis in those population subgroups at highest risk of listeriosis.

The Pouillot et al. (2015) DR model is mathematically described as follows:

$$P_{ill}(D) = 1 - \int_0^1 \exp(-r\lambda)\, f(r) dr \qquad \text{Equation 16}$$

Where:

- λ is the expected number of *L. monocytogenes* cells in one typical portion of a RTE food
- f(r): is the probability density function describing the variability of the parameter r
- $log_{10}(r) \sim Normal(\mu,\sigma)$
  - The mean (μ) is specific to each of the considered 11 population segments (see Table 12).
  - The standard deviation (σ) is assumed to be the same for all the population segments. It is summarising the variability between *L. monocytogenes* strains (σ_s) and host individual susceptibilities (σ_h). Twelve per cent of the overall variability of r (σ^2) is attributed to strain variability, the rest is for host individuals' variability within each population segment (Pouillot et al. (2015)).

In the model by Pouillot et al. (2015), the potential of a given *L. monocytogenes* strain to cause disease (i.e. strain virulence determined by a given set of transient and fixed virulence factors) were considered independent of the susceptibility of a given host to listeriosis (i.e. host susceptibility due to a given set of comorbidities and other factors impacting individual susceptibility such as genetic predisposition). To derive estimates for σ, the estimates of variability in susceptibility presented in the Food Safety and Inspection Service and Food and Drug Administration risk assessment (FSIS and FDA, 2013) were used.

$$\sigma = \sqrt{\sigma_s^2 + \sigma_h^2} \qquad \text{Equation 17}$$

Figure 10 shows the marginal DR models for each of the 11 considered population segments.

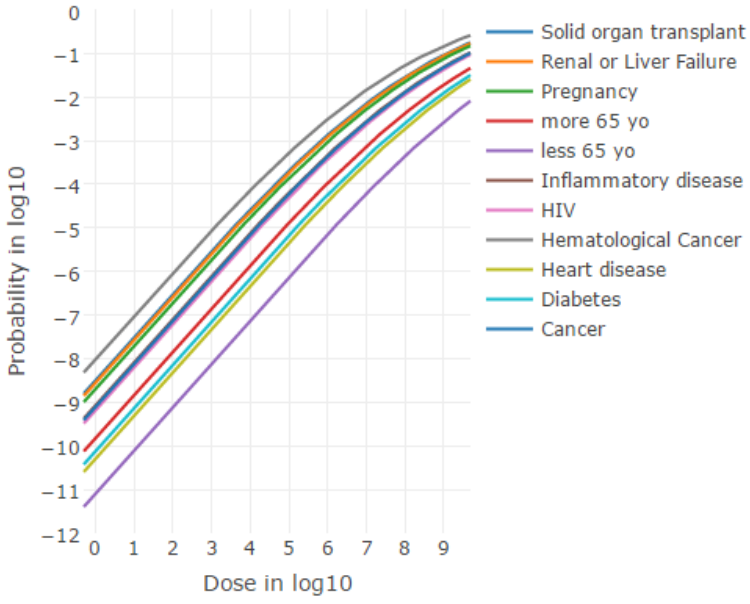


Figure 10: Dose–response models (probability of severe listeriosis cases conditional to the exposed dose) for each of the 11 population segments considered in Pouillot et al. (2015)

Table 12 provides the estimated parameter of the Pouillot et al. (2015) DR model. The Pouillot et al. (2015) and the FAO/WHO models were both applied for the under 65, over 65 and the pregnant populations in the recent *L. monocytogenes* risk assessment by Pérez-Rodríguez et al. (2017).

In 2015, data from an outbreak of listeriosis linked to milkshakes made from ice cream produced in one factory showed that contaminated products were distributed widely to the public without any reported cases, except for four cases of severe illness in persons who were highly susceptible. These data were used by Pouillot et al. (2016) to estimate the r parameter. The average level of contamination was 8 CFU/g and accounting for the uncertainty about the actual exposure dose, r was estimated within the range 1.2×10^{-7} to 5.5×10^{-7} for susceptible individuals such as those in the

outbreak. This result is in the same order of magnitude of the estimated *r* parameter by FAO and WHO (2014), 3.2×10^{-7}, from data collected during a listeriosis outbreak involving immunocompromised patients in Finland in 1998–1999. Using the model of Pouillot et al. (2015), the *r* values estimated from the ice cream outbreak data are almost 2 $\log_{10}$ higher than those based on the epidemiological data used in the original publications to estimate *r* values. These differences could be explained by a particularly virulent strain of *L. monocytogenes* present in ice cream or by outbreak investigation biases.

Table 12: The estimated parameter of the Pouillot et al. (2015) dose–response model[(a)]

Subpopulation	Geometric mean of *r*	95% variability interval of *r* [(a)]
Under 65 years old, no known underlying condition (i.e. 'healthy adult')	7.82E-15	[3.6E-18,1.7E-11]
Over 65 years old, no known underlying condition	1.47E-13	[6.7E-17,3.2E-10]
Pregnancy	1.99E-12	[9.0E-16,4.4E-09]
Non-haematological cancer	7.68E-13	[3.5E-16,1.7E-09]
Haematological cancer	9.51E-12	[4.3E-15,2.1E-08]
Renal or liver failure (dialysis, cirrhosis)	2.76E-12	[1.3E-15,6.1E-09]
Solid organ transplant	3.11E-12	[1.4E-15,6.9E-09]
Inflammatory diseases (rheumatoid arthritis, ulcerative colitis, giant cell arteritis, Crohn's disease)	8.35E-13	[3.8E-16,1.8E-09]
HIV/AIDS	6.44E-13	[2.9E-16,1.4E-09]
Diabetes (type I or type II)	7.39E-14	[3.4E-17,1.6E-10]
Heart diseases	4.96E-14	[2.2E-17,1.1E-10]

HIV/AIDS: Human immunodeficiency virus infection and acquired immune deficiency syndrome.
(a): The total variability is 1.62; the variability attributable to host individuals' differences is 0.55.

3.2.4. Summarising remarks for hazard characterisation

- *Listeria monocytogenes* is a facultative intracellular pathogen responsible for severe illnesses in humans and animal species. In addition to the systemic forms of listeriosis, a gastroenteric form exists that most likely occurs in non-immunocompromised individuals. This form of listeriosis is less well reported and its pathogenicity is less well understood.
- In the EU/EEA, during the 2008–2015 time period, bloodstream infections were the most commonly sampled and reported clinical forms of invasive *L. monocytogenes* infections (71.8% of confirmed cases), followed by meningitis (19.4% CSF samples).
- The CFR values ranged from 0.06 to 0.24 for the serogroup IIa and from 0.01 to 0.31 for the serogroup IVb. For serogroup IIa CFR values were significantly lower in the female group of age 1–44. This was also true for serogroup IVb and the CFR in the 65–74 and the ≥ 75 groups was highest and higher than the CFR in the other age and gender groups.
- There is ample evidence for a high variability regarding the virulence potential and pathogenicity of different *L. monocytogenes* isolates. Strains of only three serotypes (1/2a, lineage II; 1/2b and 4b, lineage I) have been associated with 98% of all human listeriosis. Recent studies have shown that truncation in *inlA* affects the virulence of *L. monocytogenes* but also phenotypic features such as cold adaptation. Mutations in the *inlA* gene are a frequent feature (> 30%) of *L. monocytogenes* and an attribute of some molecular subtypes such as MLST ST121 strains.
- Epidemiological data from a French strain collection (more than 6,000 isolates from both clinical specimens and food items) combined with genetic sequence information and results from animal models indicate that not more than 12 clonal complexes make up almost 80% of all isolates, and that different levels of virulence may be associated with these.
- Listeriosis is a food-borne illness, but CCs have, according to one study, been termed 'infection-associated,' 'food-associated' or `intermediate´ depending on the relative proportion of isolates isolated from clinical, food and both. 'Infection-associated' CCs are most commonly associated with CNS and MN infections as opposed to bacteraemia alone. `Food-associated´ CCs are rarely isolated from clinical samples but, when recovered from clinical specimens,

usually isolated from blood. In addition, `food-associated' CCs are more frequently associated with highly immunocompromised patients or patients showing a higher number of severe comorbidities. Based on humanised mouse models it appears that 'food-associated' CCs are less invasive (hypovirulent) than the 'infection-associated' CCs.

- A non-trivial point for interpreting the concepts of 'infection-associated' and 'food-associated' related strains is the detectability of different CCs in different matrices. Several factors related to isolation bias during enrichment and detection procedure have been reported, e.g. composition of detection media, natural microbiota in the sample, intra-species competition mediated by bacteriocins, bacteriophages or cell–cell contact.
- Despite the observed variability in their virulence potential any *L. monocytogenes* strain has the ability to result in severe human listeriosis because of the complex interaction between the pathogen, food and host.
- The probability of a single CFU to cause illness in a specific host population is reflected in the parameter *r*. This *r* parameter includes both the virulence of different *L. monocytogenes* isolates and the susceptibility of different human subpopulations. Most current DR models build on the exponential model but can be distinguished based on how the distribution of variability and uncertainty of the *r* parameter is addressed. The available *r* values range from 10^{-15} for < 65 years old without underlying conditions, to 10^{-12} for the most susceptible subpopulations, and can, when estimated for specific outbreaks with highly susceptible populations, be as high as 10^{-7}.
- A systematic literature review identified the exponential model approaches adopted by the FDA/FSIS and FAO/WHO (FDA and FSIS, 2003; FAO and WHO, 2004) as being employed in about half of the existing risk assessments.
- A lognormal-Poisson extension of the exponential model used in the FAO/WHO *L. monocytogenes* risk assessment (FAO and WHO, 2004), and the Pouillot et al. (2015) model, incorporating the virulence and susceptibility variability for 11 population groups, suggests that most human listeriosis cases are linked to the ingestion of food contaminated with medium to high (3.5 – 7.5 $\log_{10}$ CFU/serving) concentrations of *L. monocytogenes*.
- Incorporating adjustments for variability in strain virulence and host susceptibility in the lognormal-Poisson model was associated with an increase in the probability of observing listeriosis cases conditional to the exposure doses.
- Recent outbreak investigations, e.g. the US ice cream outbreak, showed that listeriosis cases in highly susceptible persons were associated with a no-growth product, with a very low average level of contamination (8 CFU/g). However, in those outbreaks it cannot be excluded that the cases were due to the exposure to high doses considering the distribution of the initial concentration and the further preparation.
- The impact of environmental factors in the food and conditions in the human host on *L. monocytogenes* virulence/pathogenicity and subsequently on health risk is not completely understood.

3.3. Evidence for exposure assessment

3.3.1. Persistence of *L. monocytogenes* strains in the food processing environment

As a saprophyte, *L. monocytogenes* effectively colonises food contact materials and other niches in food processing environments. Once residing in a niche, *L. monocytogenes* is hard to eradicate. The question is still valid if persistence is a more passive process of strains not exposed to a sufficient level of sanitation (hygiene failures) or if genetic determinants of some *L. monocytogenes* strains contribute to the phenomenon (Carpentier and Cerf, 2011). In a comprehensive study, over 2,200 environmental samples were collected following a harmonised sample scheme from 12 European food processing facilities producing RTE foods of animal origin. Food processing environments (FPEs) in each of the facilities were found positive at least once during the sampling period and the overall occurrence rate of *L. monocytogenes* was 12.6%. FPE at meat-producing facilities were found to be positive at a 4-

fold higher rate than at milk-processing facilities. A spatial evaluation of sampling schemes showed three distinct contamination scenarios: (i) widely disseminated (repeated isolation of *L. monocytogenes* from different areas and compartments); (ii) direct (repeated positive results from the same area, often close to entrances); and (iii) hotspots (infrequent positive results from single spots such as salt baths, etc. (Muhterem-Uyar et al., 2015). From this and other studies it can be concluded that *L. monocytogenes* can be detected in most FPEs over time to a varying degree, and a total absence of *L. monocytogenes* in the FPE cannot be expected. This highlights the need for appropriate sampling programmes and corrective actions to prevent *L. monocytogenes* from being transmitted from in-house sources to the product. One approach envisaged in the USA for *L. monocytogenes* control is the 'seek and destroy' concept (Malley et al., 2015).

Listeria monocytogenes can persist for months or even years in various environmental niches, including chilled food plants (Lundén, 2004; Møretrø and Langsrud, 2004; Keto-Timonen et al., 2007; Schmitz-Esser et al., 2015). Survival in nature seems to be dependent on altitude and humidity (Linke et al., 2014). Persistence could be due to high adaptive capacity against physical–chemical factors and due to other genetic determinants increasing survival capacity. Studies on the biofilm-forming capacity of *L. monocytogenes* do not result in a conclusive picture. Although a gene product has been identified that goes with biofilm formation in *L. monocytogenes* it does not seem that this bacterium is a very effective biofilm producer (Barbosa dos Reis-Teixeira et al., 2017). A limitation of many biofilm studies is that they are usually performed in highly artificial experimental settings (plastic microtitre plates) or that the phenotype strongly varies, with some strains producing biofilms and others not. Regarding the physical–chemical factors, to withstand a wide range of temperatures (2–45°C), *L. monocytogenes* changes its fatty acid composition of cell membranes (Annous et al., 1997). *Listeria monocytogenes* is able to tolerate a pH of 4.1 to 9.6 (Lungu et al., 2009). Some evidence exists that persistent strains may cope better with acidic conditions than non-persistent strains do (Lunden et al., 2008). The acid tolerance response (ATR) system allows the survival of *L. monocytogenes* at low pH values up to 5.5. In addition, through the activation of the ATR system bacterial cells can also become adapted to severe acid stress (pH 3.5; (O'Driscoll et al., 1996)). Moreover, the glutamate decarboxylase (GAD) system is also responsible for acid resistance (Cotter et al., 2001).

Listeria monocytogenes is also well adapted to osmotic stress, particularly to high concentrations of salt (Gandhi and Chikindas, 2007). As many as 21 functionally active osmoprotective systems have been described up to now, with many more to come (reviewed by Burgess et al. (2016)). Two groups of proteins were distinguished, namely salt shock proteins and stress acclimation proteins (Duche et al., 2002). To name two mechanisms, *L. monocytogenes* cells accumulate osmo-protectants such as glycine betaine, proline betaine, acetyl carnitine, carnitine, butyrobetaine and 3-dimethylsulphoniopropionate which protect the cells against high salt concentrations (Bayles and Wilkinson, 2000). Furthermore, there is a two-component regulatory system consisting of a homologous KdpE protein (an ATPase with high affinity for potassium), *inter alia*, which receives potassium under salt stress into the cells. The high potassium concentrations in the cells activate the two stress-regulating genes *kdpE* (encodes the response regulator) and the downstream gene *orfX* (Walderhaug et al., 1992; Brondsted et al., 2003). Along with osmotic stress, desiccation stress might have an important impact on *L. monocytogenes* growth. *L. monocytogenes* positive samples from food products with low water activity (a_w) have been repeatedly reported. In comparison to other stress-related factors, there is relatively little knowledge on desiccation tolerance of *L. monocytogenes* available in the scientific literature (Burgess et al., 2016). Cold adaptation in *L. monocytogenes* is a particular feature of this facultative pathogen and often associated with osmotic stress tolerance. *L. monocytogenes* possesses small, highly homologous protein members of the cold shock protein (Csp) family but there are other molecular mechanisms described that contribute to the cold adaptation potential (Tasara and Stephan, 2006). Cold shock proteins and cold acclimation proteins are temperature-induced (Bayles et al., 1996). Furthermore, *L. monocytogenes* is able to accumulate cryoprotectants such as glycine betaine and carnitine at refrigeration temperatures (Bayles and Wilkinson, 2000; Angelidis and Smith, 2003). Cold adaptation makes *L. monocytogenes* particularly capable of surviving in food stored in cold chains of modern food production and retail systems (see Section 3.3.3.).

Against this background, scientific studies are being performed to better understand the genetic determinants that contribute to the persistence phenomenon. It is well established that some clones of *L. monocytogenes* tolerate higher concentrations of disinfectants. A transposon (Tn6188) was

shown to confer tolerance against quaternary ammonium compounds (Muller et al., 2013). Other plasmid-based genetic elements such as the bcrABC cassette were shown to be associated with increased persistence (Elhanafi et al., 2010). Of interest for persistence is the hypervariable genetic hotspot lmo0443-lmo0449 that appears to play a role in stress response (Ryan et al., 2010). So far, three distinct insert sequence types are known for the genetic locus mentioned above: stress survival islet 1 (SSI-1), lin0464-lin0465 and LMOf2365_0481 (Ryan et al., 2010). SSI-1 consists of five distinct genes, namely lmo0444, lmo0445, pva (lmo0446), gadD1 (lmo0447) and gadT1 (lmo0448) (Ryan et al., 2010). The function of the individual genes of the islet was previously understood as follows: pva has significance for the tolerance of *L. monocytogenes* to bile (Begley et al., 2005); genes gadD1 and gadT1 are involved in the GAD system thus affecting the survival of *L. monocytogenes* in mildly acidic environments (Cotter et al., 2005). In fact, it was demonstrated that SSI-1 positively affects bacterial growth under salt and acid stress. The regulatory mechanisms of SSI-1 are not conclusively understood. However, the alternative stress sigma factor SigB assumes a regulatory impact while the central virulence regulator PrfA exerts no influence on the insertion SSI-1. Furthermore, lmo0445 appears to exert a regulatory function on the other four genes of the islet (Ryan et al., 2010).

The insert sequence type lin0464-lin0465 is a fragment of 2.2 kb which contains two genes: lin0464 and lin0465 are homologues of *L. innocua* genes lin0464 and lin0465 (Hein et al., 2011). This insert is extremely prevalent in *L. monocytogenes* MLST 121, a hypovirulent subtype that is often isolated from FPEs (Rychli et al., 2014). The smallest of the three insert types is LMOf2365_0481 because it has a size of 713 bp (base pairs). Of all three inserts, the least is known about LMOf2365_0481 and its function remains to be elucidated. An interesting question concerns the distribution of marker genes for persistence in the *Listeria* population. Attempts have been undertaken to establish an SNP-based system to distinguish between persistent and non-persistent strains (Stasiewicz et al., 2015). Møller Nielsen et al. (2017) tried to study the marker gene diversity in 1,143 *L. monocytogenes* strains of clinical and food-borne origin by an NGS approach. In their report, they summarised that abundance of putative markers of resistance to detergents, disinfectants and antiseptics, e.g. via efflux mechanisms, was close to 20%. However, the authors emphasised that the presence or absence of genes promoting a persistent phenotype was not found to be pertinent in their strain set. Their study did not focus on the accessory genome, which by definition comprises genes mostly located on mobile elements, which may not be present ubiquitously across the *L. monocytogenes* population. The analysis of the accessory genome is important as it has been recently shown that conservation of the accessory genome might be associated with persistence (Fagerlund et al., 2016). Genes on plasmids or other mobile elements such as transposons will make a significant contribution to the variation in biology seen between isolates and therefore should be a rich source for the discovery of polymorphisms associated with persistence and other features. It should be noted that sequence analysis is not enough to fully understand the regulatory background of the persistence phenomenon in *L. monocytogenes*. Expression of gene markers for persistence (Mazza et al., 2015) or proteome analysis (Rychli et al., 2016) have recently appeared promising for predicting persistence phenotypes.

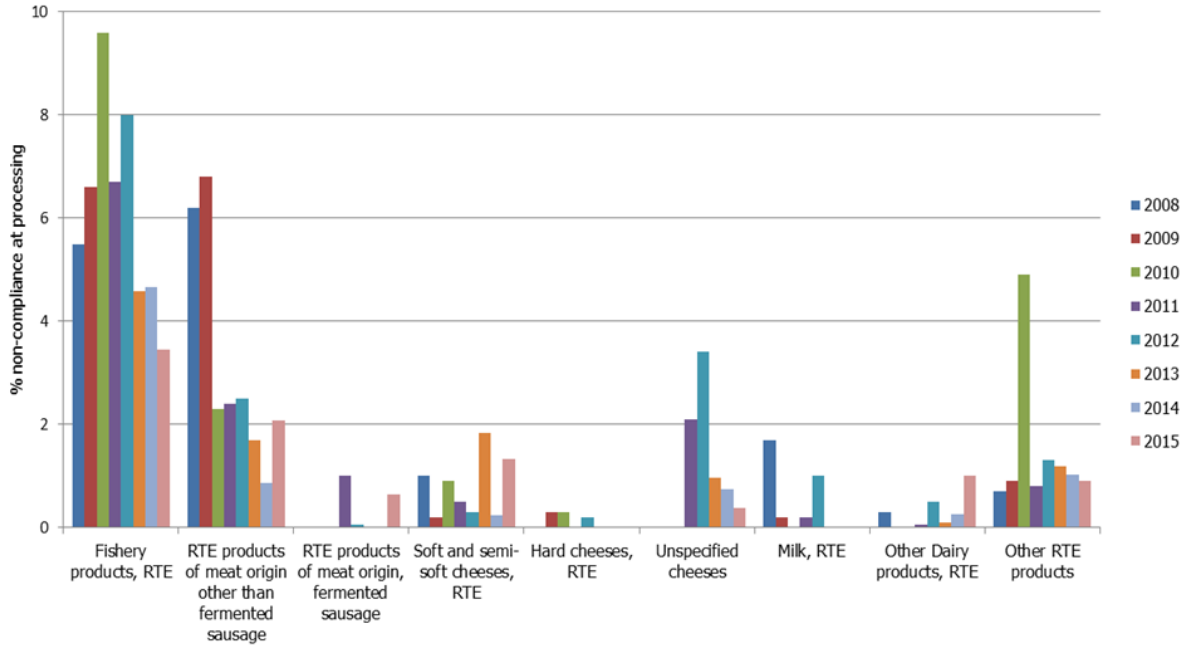
3.3.2. Prevalence and concentration of *L. monocytogenes* in RTE foods

EFSA monitoring data

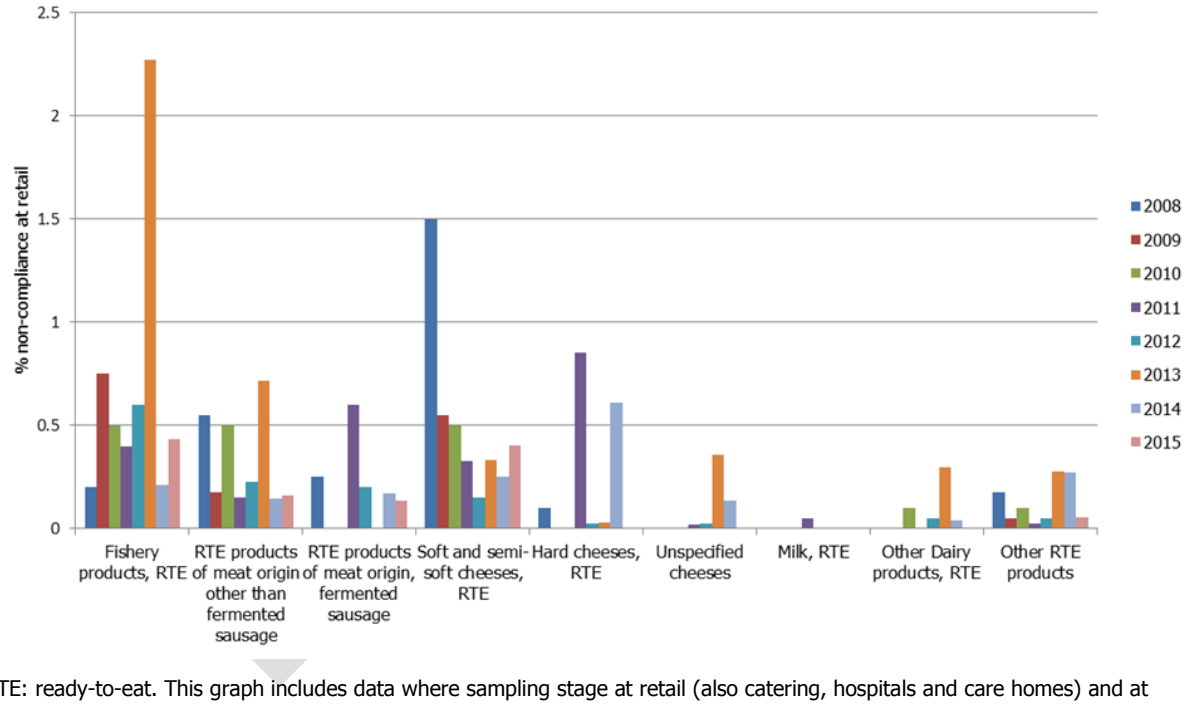
Compliance of different RTE food subcategories with the *L. monocytogenes* FSC in 2008–2015 is presented in Figure 11. The figure includes monitoring data according to sampling stage, for the relevant food types at retail (also catering, hospitals and care homes) and at processing (also cutting plants). Data collected at 'unspecified' sampling stages are included in the data reported at retail. The apparently higher proportion of non-compliance at processing is at least partly explained by the application of the different limit of FSCs for retail and processing (see footnote to Figure 11).

Considering the sampling stage of **processing**; apart from 2008 and 2009, 'RTE fishery products' was the food category with the highest level of non-compliance. It ranged from 3.5 to 9.6% of single samples. For 'RTE products of meat origin other than fermented sausage' and 'RTE products of meat origin, fermented sausage' the level of non-compliance ranged between 0.9 and 6.8%, and 0 and 0.6%, respectively. In the case of cheese, 'soft and semi-soft cheese' (0.2–1.8%) overall showed a higher level of non-compliance than 'hard cheese' (0–0.3%). For 'unspecified cheese,' 'milk, RTE' and 'other RTE dairy products' respectively 0.4–3.4%, 0–1.7%, and 0–1% single samples were non-compliant.

(a)



(b)



RTE: ready-to-eat. This graph includes data where sampling stage at retail (also catering, hospitals and care homes) and at processing (also cutting plants) have been specified for the relevant food types. Data collected at the 'unspecified' sampling stage are included in the data reported at retail. The category 'other RTE products' includes RTE food other than: 'RTE fishery products,' 'soft and semi-soft cheese,' 'hard cheese,' 'unspecified cheese,' 'other RTE dairy products,' 'milk,' 'RTE products of meat origin other than fermented sausage,' 'RTE products of meat origin, fermented sausage.' For the non-compliance analysis of samples collected at the processing stage, the food safety criterion of 'absence in 25 g' was applied, except for samples of hard cheese and fermented sausage that were assumed to be unable to support the growth of *L. monocytogenes* and for which the criterion of '≤ 100 CFU/g' was applied. For the non-compliance analysis of samples collected at the retail level, the FSC of '≤ 100 CFU/g' was applied. Only information on the main RTE food categories (RTE fishery products, RTE cheese and RTE meat products) is included in this graph.

Figure 11: Proportion of single samples at processing (a) and retail (b) non-compliant with EU *Listeria monocytogenes* food safety criteria, 2008–2015

Considering the **retail** sampling stage, 'RTE fishery products' had the highest level of non-compliance in 2013 (2.3% of single samples), while for other years it was below 0.8%. For 'RTE products of meat origin other than fermented sausage' and 'RTE products of meat origin, fermented sausage' the highest levels of non-compliance were 0.7% (in 2013) and 0.6% (in 2011). In the case of 'soft and semi-soft cheese' the level of non-compliance was below 0.6%, except in 2008 (1.5%). For 'hard cheese' the level of non-compliance was below 0.3%, except in 2011 (0.9%) and 2014 (0.6%). For 'unspecified cheese,' data have only been reported since 2011 with the highest level of non-compliance in 2013 (0.4%). For 'milk, RTE' the level of non-compliance was below 0.1% for all years. This was also the case for 'other RTE dairy products', except in 2013 (0.3%). Between 0.05 and 0.3% of single samples of 'other RTE products' were found to be non-compliant.

Although non-compliance at retail of less than 1% may be considered low, this may still correspond to many servings containing more than 100 CFU/g when total consumption is taken into account.

EU-wide prevalence of *Listeria monocytogenes* in RTE foods

The estimates of prevalence across the EU, as derived from the BLS conducted in 2010 and 2011, of *L. monocytogenes*-contaminated fish, meat and cheese samples, and of the proportion (%) of samples with *L. monocytogenes* counts exceeding the level of 100 CFU/g (among the sampled categories of RTE foods, as described above) can be found in Table 13.

Table 13: Prevalence (%) of *Listeria monocytogenes*-contaminated fish, meat and cheese samples, and proportion (%) of samples with *Listeria* counts exceeding the level of 100 CFU/g at the time of sampling (for fish only) and at the end of shelf life, in the EU, 2010–2011 (from EFSA (2013))

Product and subtype	Number of samples	At sampling		At end of shelf life	
		Prevalence with 95% CI (%)	Proportion > 100 CFU/g with 95% CI (%)	Prevalence with 95% CI (%)	Proportion > 100 CFU/g with 95% CI (%)
Total fish	**2,994**	**10.4 (9.1–11.7)**	**1.0 (0.7–1.4)**	**10.3 (9.1–11.6)**	**1.7 (1.3–2.3)**
Cold-smoked fish	599	17.4 (14.2–21.1)	1.7 (0.9–3.2)	16.0 (13.2–19.3)	2.0 (1.1–3.6)
Hot-smoked fish	525	6.3 (4.4–8.9)	1.3 (0.6–2.8)	6.7 (4.7–9.3)	1.7 (0.9–3.3)
Unknown smoked fish[(a)]	1,625	8.8 (7.3–10.5)	0.6 (0.3–1.2)	9.1 (7.6–10.9)	1.8 (1.2–2.6)
Gravad fish	245	12.2 (8.7–17.0)	0.8 (0.2–3.2)	12.2 (8.6–17.1)	0.8 (0.2–3.2)
Total meat	**3,470**	**ND**	**ND**	**2.07 (1.63–2.64)**	**0.43 (0.25–0.74)**
Total cheese	**3,393**	**ND**	**ND**	**0.47 (0.29–0.77)**	**0.06 (0.02–0.24)**

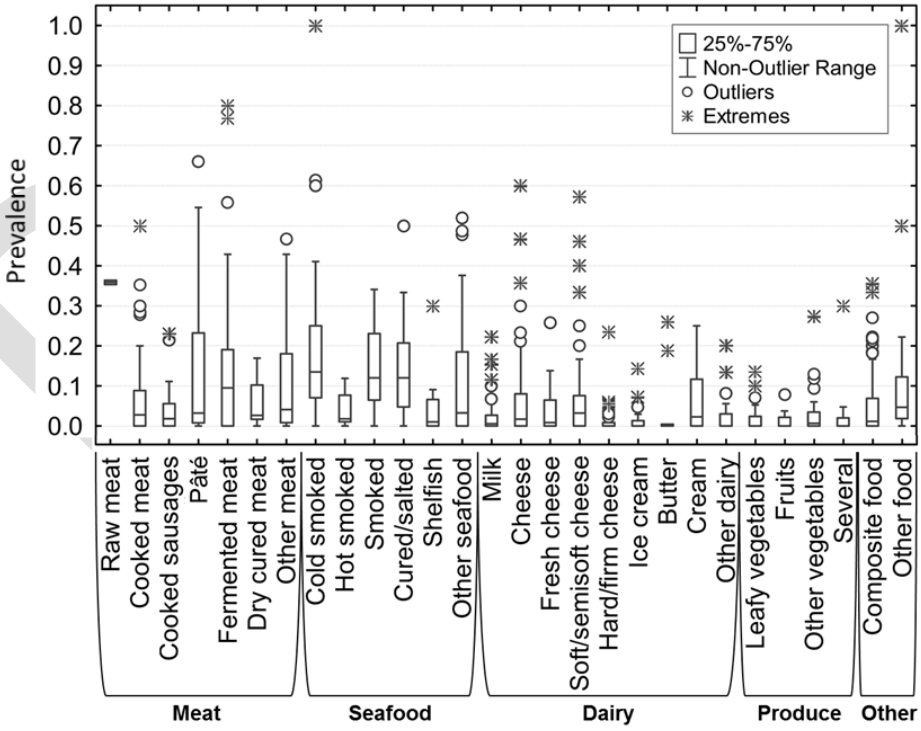
CI: confidence interval; ND: not determined.
Portugal did not participate in the baseline survey and one non-Member State, Norway, participated. Norway is not included in the EU prevalence estimation analysis. Prevalence was based on combined detection and enumeration methods results. A food sample was considered positive if *L. monocytogenes* was detected by at least one of either the detection or the enumeration method, (i.e. a sample was regarded as positive when either the detection test result was positive and/or the enumeration test result was positive, i.e. having a count of at least 10 CFU/g). The survey specifications defined particular subsets of food products to be sampled, specifically (i) RTE fish which were hot-smoked or cold-smoked or gravad, were not frozen, and were vacuum or modified atmosphere packaged; (ii) RTE meat products which had been subjected to heat treatment, and were then vacuum or modified atmosphere packaged; (iii) RTE soft or semi-soft cheese, excluding fresh cheese. This category includes smear-ripened, mould-ripened, brine-matured or otherwise ripened, cheese made from raw, thermised or pasteurised milk of any animal species. The cheese could be packaged, or unpackaged at retail but packaged at the point of sale for the consumer. Only packaged and intact (sealed) packages, packaged by the manufacturer, were to be collected for sampling. However, in the case of cheese and meat products, products packaged at the retail outlet could also be collected for sampling.
(a): fish which may have been hot- or cold-smoked.

The EU prevalence estimate in fish samples at the time of sampling was 10.4% and at the end of shelf life was 10.3%. The EU-level estimate of the proportion of samples with *L. monocytogenes* counts exceeding the level of 100 CFU/g at sampling was 1.0% while for fish samples at the end of shelf life it was 1.7%. Among meat products, the EU prevalence of *L. monocytogenes*-contaminated samples at the end of shelf life was estimated at 2.07% while the EU-level proportion of samples with *L. monocytogenes* counts exceeding 100 CFU/g was estimated at 0.43%. The EU estimate of prevalence of *L. monocytogenes*-contaminated cheese samples at the end of shelf life was 0.47% while the EU-level estimate of proportion of samples with *L. monocytogenes* counts exceeding 100 CFU/g was 0.06%.

Prevalence of *Listeria monocytogenes* in RTE foods from literature studies

The extensive literature search performed by Jofré et al. (2016) on the occurrence and levels of contamination of *L. monocytogenes* in a wide range of RTE foods covering the 1990–2015 period yielded 308 records eligible for data extraction. About 90% of the studies were surveys of naturally contaminated RTE foods with quantification of prevalence and/or levels of *L. monocytogenes* as (one of) the purpose/s of the study. Altogether, the category 'dairy products' was included in most records (N = 139), followed by 'meat products' (N = 110), 'seafood' (N = 79), 'composite food' (N = 62, including meals such as pasta- and rice-based salads, pre-cooked chilled foods, sandwiches, sushi, pastry and desserts), 'produce' (N = 58) and 'other types of products' (N = 16, including egg products and other un-specific/non-described 'RTE products' in general). Some studies deal with more than one food category, therefore the sum of records is higher than the 308 reviewed studies.

Prevalence data were available for 778 outcomes, i.e. individual-item survey results. The RTE food category with most prevalence data were dairy products (N = 276), followed by meat products (N = 173), seafood (N = 151), other products (e.g. composite products of raw materials from different categories; N = 104) and fresh produce (N = 74). In total, *L. monocytogenes* was detected in 78.1%, 70.5%, 51.8%, 36.5%, and 47.1% of the studies dealing with seafood, meat products, dairy products, produce and other products, respectively. Figure 12 shows the box-plot representation of the *L. monocytogenes* prevalence of each RTE food subcategory.



Median value is indicated by the line within the interquartile box. Outliers (○) and extreme (✳) values correspond to values at 1.5- and 3-fold the interquartile range, respectively from the 75th percentile.

Figure 12: Box-plot showing the *Listeria monocytogenes* prevalence of ready-to-eat (RTE) foods by subcategory

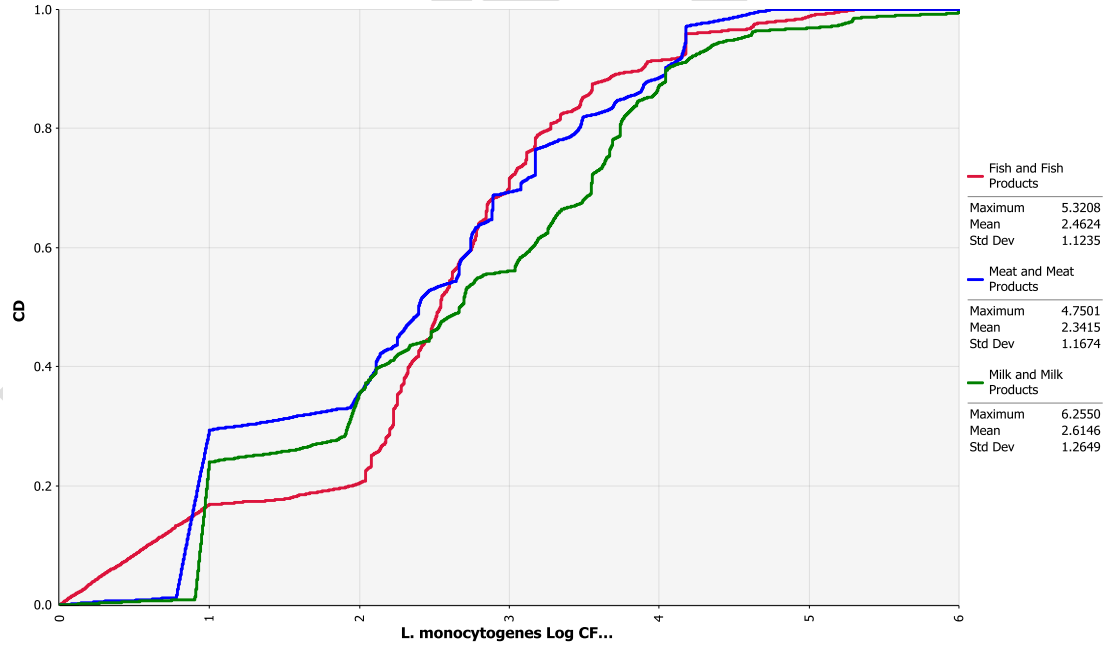
In all subcategories, the distribution of the prevalence values was asymmetric, with several outliers as well as extreme values. For the whole period, the median of the prevalence was below 10% for almost all subcategories, except for fermented sausage (10%), cold-smoked fish (13%), smoked fish (either cold- or hot-smoked; 12%) and cured/salted fish (12%).

Semi-quantitative data about *L. monocytogenes* levels (e.g. grouped in concentration ranges or above/below 100 CFU/g or ml) was provided in 244 studies. The highest number of semi-quantitative data points has been recorded for meat products (N = 62). Quantitative data were obtained for only 14 RTE product types. More information can be found in Jofré et al. (2016).

RASFF data

Based on the criteria described in Section 2.1.2, the total number of RASFF notifications analysed for the concentration of the pathogen were 130 for the RASFF product category 'fish and fish products,' 126 for the RASFF product category 'milk and milk products' and 81 for the RASFF product category 'meat and meat products other than poultry.' In order to include notifications reporting concentrations less than the detection limit (i.e. < 10 CFU/g) in the analysis, data were formed as CDF and the statistical analysis was performed using the Monte Carlo simulation (10,000 iterations).

Figure 13 presents the CDF of the *L. monocytogenes* concentration for RASFF food product category 'fish and fish products,' 'milk and milk products' and 'meat and meat products other than poultry' reported in all RASFF notifications during the years 2008–2016. For example, the concentration is over 2 $\log_{10}$ CFU/g in approximately 80%, 65% and 65% of 'fish and fish products,' 'meat and meat products,' and 'milk and milk products,' reported in RASFF notifications, respectively. The average concentrations were 2.61, 2.46 and 2.34 $\log_{10}$ CFU/g for 'milk and milk products,' 'fish and fish products' and 'meat and meat products other than poultry,' respectively. The highest maximum concentrations were 6.25 $\log_{10}$ CFU/g, 5.32 $\log_{10}$ CFU/g and 4.75 $\log_{10}$ CFU/g, respectively.



The empirical cumulative distribution function is a step function that jumps up by 1/n at each of the n data points. Its value at any specified value of the measured variable is the fraction of observations of the measured variable that are less than or equal to the specified value. Example: the red curve shows that concentration has a probability of 20% to be less or equal to 2 $\log_{10}$ CFU/g.

Figure 13: Empirical cumulative distribution function of the reported *Listeria monocytogenes* concentration in RASFF notifications (2008–2016) for 'fish and fish products' (N = 130), 'milk and milk products' (N = 126) and 'meat and meat products other than poultry' (N = 81)

3.3.3. Consumption and food handling

Consumption and food handling can impact on human listeriosis risk since exposure may increase with increased consumption of RTE foods with a high likelihood of being contaminated, and with improper food handling that may increase the spread and growth of *L. monocytogenes*. Thus, changes in consumption patterns or differences in food handling among population groups have been proposed as potential drivers for changes in the incidence rates of listeriosis (Yang et al., 2006; ACMSF, 2009b).

Consumption

In Germany, a national case–control study of sporadic non-pregnancy-associated listeriosis cases between 2012 and 2013 identified consumption of cold cooked sausages, and the consumption of packaged cheese and pre-sliced cheese as food-related risk factors (Preussel et al., 2015). A retrospective case–control study of listeriosis patients in England aged over 60 identified that cases were more likely than controls to report the consumption of cooked meats (beef and ham/pork, but not poultry), cooked fish (specifically smoked salmon) and shellfish (prawns), dairy products (most noticeably milk but also certain cheeses), and mixed salads. They were less likely than controls to report the consumption of other forms of seafood, dairy spread, other forms of dairy products, sandwiches, and fresh vegetables (Gillespie et al., 2010). Based on the general UK population over 65 years old, another study concluded that it was not possible to determine any particular factor in the shopping and consumption patterns for people over 65 years old that was likely to increase their risk of listeriosis. However, compared with all consumers there was a tendency to eat more homemade, chilled and fresh (not frozen) foods and to consume more food cold than hot (ACMSF, 2009b). According to a UK discussion paper on risk factors among older consumers (ACMSF, 2009a), there is a need to better understand how the dietary practices of people aged 60 years and over are affected by ageing and how this may be linked to a potentially increased exposure to *L. monocytogenes*.

Serving size

Statistical parameters and probability distribution parameters for serving size of the target food subcategories and three population groups in the EU were developed by Pérez-Rodríguez et al. (2017). The data for pregnant women were based only on a single available study and these limited data were not used in that risk assessment. Instead, serving sizes for pregnant women were assumed to be similar to the general adult population. More information on serving sizes and the total number of servings in the 28 Member States across the EU can be found in Pérez-Rodríguez et al. (2017).

For the purpose of the present Scientific Opinion, serving sizes for different age and gender groups were also estimated based on national surveys carried out between 1997 and 2012 and available in the EFSA consumption database. In Table 14 the mean of the mean serving sizes reported in these surveys are shown, illustrating the differences in serving sizes between different age groups for six subcategories of RTE foods.

Table 14: Mean of the mean serving sizes (g) in the most recent national surveys from the EFSA food consumption database

Age groups (years)	Fish products				Meat products						Cheese	
	Gravad fish[(a)]		Smoked fish		Cooked meat		Heat-treated sausages		Pâté		Soft and semi-soft cheese	
	F	M	F	M	F	M	F	M	F	M	F	M
1–4	25	-[(b)]	26	21	22	23	38	44	19	22	21	20
5–14	47	68	54	56	31	32	54	63	28	29	27	43
15–24	132	101	56	57	39	51	68	90	36	49	40	43
25–44	95	151	64	78	42	53	61	79	41	53	48	45
45–64	96	134	61	87	42	53	63	78	41	49	46	44
65–74	144	129	60	58	40	42	55	70	31	44	32	40
≥ 75	154	132	49	66	30	42	63	61	33	38	36	41

F: female; M: male.
(a): In the gQMRA model it was assumed that the serving size of gravad fish is the same as that of smoked fish.
(b): There were no servings in this group.

The means of the median, 25th percentile and 75th percentile are shown in Appendix G. The largest mean of the mean serving sizes were found for gravad fish followed by smoked fish and heat-treated sausages. It should be noted that differences are sometimes small and that the data for gravad fish are based on surveys from only one Member State.

The numbers of yearly servings per age and gender are presented in Appendix G and the total number of servings in Table 15. Cooked meat and heat-treated sausages were the subcategories with the most consumed servings per person and year and for meat products the number of servings was in general greater for males than for females (Table 15 and Appendix G). For the other food subcategories gender differences in consumption frequency varied by age (Appendix G). The pattern with the highest number of servings associated with cooked meat and heat-treated sausages are reflected in the total number of servings in the EU/EEA (Table 15). Since these figures reflect the size of the populations, in general fewer total numbers of servings of RTE foods are consumed by age groups over 65 years than by those between 25 and 65 years old. In the BLS 0.43% of RTE meat and meat products contained concentrations of *L. monocytogenes* above 100 CFU/g (Table 13). Under the assumption that this result reflects the corresponding RTE food category considered when estimating the frequency of consumption, this would translate to approximately 55 million such contaminated servings being consumed by the over 75 age group per year in the EU/EEA.

Table 15: Mean number of servings (in millions) per year in the EU/EEA based on the mean number of servings per day estimated from the most recent national surveys (1997–2012) in the EFSA food consumption database and population data from 2015

Age groups (years)	Gravad fish[(a)]		Smoked fish		Cooked meat		Heat-treated sausages		Pâté		Soft and semi-soft cheese	
	F	M	F	M	F	M	F	M	F	M	F	M
1–4	7	0	271	306	749	864	1,000	982	591	650	232	202
5–14	13	5	222	225	2,488	2,778	2,444	2,838	958	1,211	475	475
15–24	43	73	398	263	2,788	4,055	1,642	2,713	671	1,057	679	593
25–44	253	164	831	933	8,449	11,252	4,696	7,659	1,644	2,892	2,296	2,033
45–64	337	314	1,389	1,567	9,213	11,563	5,287	8,027	1,589	2,735	2,455	2,558
65–74	287	189	1,006	994	3,869	4,001	2,049	2,402	782	1,076	1,049	1,054
≥ 75	88	39	1,586	1,574	3,565	2,780	2,021	1,990	1,231	1,177	1,334	1,183
Mean (all ages)	1,028	784	5,703	5,862	31,121	37,293	19,139	26,611	7,466	10,798	8,520	8,098

F: female; M: male.
(a): In the gQMRA model it was assumed that the number of servings of gravad fish is 22.3% of those of smoked fish.

Consumer food handling

Consumer food handling practices expected to have the largest impact on exposure and risk are those that can lead to contamination of RTE foods, e.g. cross-contamination to unpackaged foods in the refrigerator, or to actions that may allow increased growth, i.e. improper storage temperatures and times. In one risk assessment of *L. monocytogenes* in deli meats up to a million-fold increase in risk due to consumer handling was estimated, and storage practices appeared to be more important in terms of risk than cross-contamination (Yang et al., 2006).

According to a review of consumer food safety studies from 1993–2014, in the majority of studies (83%) survey methods were used, some (29%) also used observational methods, mostly by determination of the operational temperature in refrigerators, and a few (12%) used focus groups (Evans and Redmond, 2014). Thus, the majority of information on consumer behaviour is based on self-reporting via questionnaires or interviews where it may be difficult to know how responses relate to actual behaviour, since it is not uncommon that there is a difference between what is known about handling and what is done in practice (Redmond and Griffith, 2003). Direct observation methods allow assessment of actual behaviour but may be subject to bias since consumers may change their behaviour in response to the 'experimental' situation. The review of Evans and Redmond Evans and Redmond (2014)covered studies related to behavioural risk factors for listeriosis in the home and supports the conclusion that consumer handling related to storage and other self-reported practices are risk factors (Evans and Redmond, 2014). This could be due to lack of consumer knowledge, consumer attitudes or understanding. Differences were observed between different groups, for instance categorised by gender (e.g. Alibabic et al. (2012) and Brennan et al. (2007)) or

education/training (e.g. Brennan et al. (2007)). In relation to risk factors, the review indicated that consumer understanding of use-by dates is often lacking and in practice adherence to these may be very variable. In relation to storage of food products in refrigerators there were generally positive attitudes for the need for correct temperatures but a large proportion of consumers did not know the recommended temperatures.

The reported differences between groups in the population are commonly presented and interpreted by separating consumers into various groups characterised by narrative labels (Kennedy et al., 2005b; Kendall et al., 2013), where factors such as age, socioeconomic status (married, divorced, unemployed), general education, home economics training (Brennan et al., 2007), cognition (Evans and Redmond, 2016b), psychology (Fischer and Frewer, 2008) have been related to behaviour. Significant life-stage events may have an impact on food handling behaviour, e.g. the death of spouse/partner, divorce or separation. Brennan et al. (2007) categorised males over 65 years of age who were widowed, divorced or separated as one of four high-risk groups in terms of microbiological food safety in Ireland. The other three risk groups were single 18–34-year-old non-student males, without home economics training in school; 18–24-year-old female homemakers, without home economics training; and, perhaps unexpected, > 45-year-old female homemakers with home economics training. Possible explanations put forward for the last group was that best practice had changed since this group received training or over-confidence in their own judgement. Several studies have identified young and elderly males as risk groups in terms of knowledge, attitudes and behaviour (e.g. McCarthy et al. (2007) and Rossvoll et al. (2013)).

Several studies have highlighted the diversity of elderly and other risk groups and that differences in food handling practices may be great within different narratively characterised consumer groups (e.g. divorced, unemployed) and also between studies from different countries. Indeed, the group over 60 years old may include those who are a generation apart, with or without underlying health conditions, in addition to other socio-demographic differences (ACMSF, 2009a). This socio-demographic diversity among cases is well captured and very illustrative in the description of five anonymous listeriosis cases in the UK (ACMSF, 2009a). The existence of national differences was illustrated in a study reporting that Belgian consumers less frequently stored their fresh produce in a refrigerator and did so for a shorter time than Spanish consumers (Jacxsens et al., 2015). There is also a lack of food handling studies for risk groups other than the elderly, e.g. pregnant women (Pereboom et al., 2014) and other specific vulnerable groups.

Since the increase in the number of listeriosis cases has been associated with the older population and this group often also includes other vulnerable groups, the food handling practices of this group are of particular interest. In addition to socioeconomic factors a number of ageing-related effects may impact on how the elderly handle food. For instance 'deterioration of oral health, eyesight, hearing, reduction in mental stimulation and social interaction opportunities; reduction in physical mobility (both personal and transport); chronic physical deterioration/pain (including arthritis and osteoporosis); early stage dementia/memory-related problems' (ACMSF, 2009a). Based on limited data, factors that were identified in the UK population over 65 years old that may contribute to increased *L. monocytogenes* exposures were keeping food beyond the use-by dates or not keeping them refrigerated at suitable temperatures (ACMSF, 2009b). A more recent study supports that conclusion and reported that knowledge among older consumers about appropriate refrigerator temperatures was poor and ownership of thermometers was low (Evans and Redmond, 2016b). Although the majority of older adults may store leftover chilled food in the refrigerator, one study reported that 78% of older adults kept sliced cooked meat uncovered in the refrigerator (Terpstra et al., 2005), and in another study 38% reported they would store leftover food out on the counter (Almanza et al., 2007). A combined use of observation, self-reporting and microbiological analysis was employed to identify risk factors among the group of consumers over 60 years old in the UK (Evans and Redmond, 2016b). Forty-one per cent of foods in home refrigerators were beyond the use-by date, and of these 11% were RTE foods commonly associated with listeriosis. Of opened RTE foods, 66% had been, or were reportedly intended to be, stored beyond the recommended two days after opening. Refrigeration temperatures were above the 5°C recommended storage temperature in the UK in 50% or more of storage areas, and older refrigerators operated at significantly higher temperatures. In addition, *L. monocytogenes* was isolated in 2% of the kitchens. In contrast, concern about and understanding of the concept of use-by dates was reported among older adults but studies proving adherence or observational data to support this is generally lacking (Evans and Redmond,

2014). A US study among senior-aged women and women of child-bearing age reported that opened packages were often being stored for longer than recommended and that interpretation of the labels was highly variable but both age groups considered use-by more helpful than other types of labelling (Lenhart et al., 2008). One study in the UK indicated that the failure to follow use-by dates was due to the difficulty of reading the labels (Johnson et al., 1998). It should be highlighted that comparatively few studies of 'the over 60s' exist and from few countries. Evans and Redmond (2014) reported that only 7% of the consumer food safety studies reviewed included data for older adults, i.e. over 60 years old.

Another overview of food safety studies with focus on reported differences between older (> 60 years) and younger consumers carried out as a follow-up of the UK ad hoc report concluded that it is not known whether knowledge levels differ with generations or have changed as people age, and, if knowledge levels have changed, why that change may have occurred (ACMSF, 2009a).

In conclusion, knowledge gaps make it difficult to conclude in a quantitative manner on the range of food handling behaviours in different risk and age groups and on how this may contribute to trends of listeriosis. However, based on the available studies, unsafe practices are not uncommon, > 10%, among the elderly and can have a potential impact on the occurrence of listeriosis cases. There is a wide variation within broadly defined consumer groups and it is thus problematic to generalise about food handling behaviours of these groups. The majority of studies about food handling are from a few countries which contribute some uncertainty concerning the generalisability of the results presented. The extent of different behaviours among risk groups may vary to the same extent that socioeconomic factors, traditions and types of food vary between Member States. There is a need for better information on human listeriosis cases in terms of socioeconomic–demographic data.

Storage temperature of RTE foods in retail and domestic refrigerators

The logistic chain of RTE foods includes storage at the production point or distribution centres, transportation, retail and domestic storage. The temperature during the first steps of the chain are in most cases satisfactorily controlled (Afchain et al., 2005). In contrast, conditions at the retail level are out of manufacturers' direct control and often deviate from legislated temperature limits while temperature control is completely in the hands of the consumer at domestic level. In general, the temperature during storage at retail is lower than during domestic storage (EFSA BIOHAZ Panel, 2008). Available survey studies on retail storage temperatures in France, Slovenia, Greece and Finland reported a mean temperature ranging from 2.7 to 5.6°C (Pierre, 1996; Afchain et al., 2005; Derens et al., 2006; Likar and Jevsnik, 2006; Koutsoumanis et al., 2010; Lunden et al., 2014). Storage temperature, however, may vary between retail cabinet types as well as between positions in the cabinet. Maximum temperatures in cabinets were generally in the most exposed (to ambient) areas and minimum temperatures are located in the least exposed areas (Evans et al., 2007). In addition, Koutsoumanis et al. (2010) reported a variation of temperature with time in retail cabinets in which periodic up-shifts of temperature may occur due to the defrost system of the refrigerators. This may affect microbial growth depending on the food, the direction and duration of the temperature shifts and the *L. monocytogenes* strain.

RTE foods with extended shelf life may be stored in a domestic refrigerator for long time. In addition, consumers do not always respect the instructions on time and temperature of storage indicated on the label (Marklinder and Erikkson, 2015). Domestic refrigerator temperatures can therefore have a significant effect on the risk of listeriosis. Table 16 presents data from 23 survey studies on domestic refrigerator temperatures from eight European countries. The data are presented in such a manner as to facilitate comparison between surveys, although this is not always possible due to the use of different parameters and temperature ranges in the reporting of the data. Of the 16 surveys for which a mean temperature was given, this ranged from 5 to 8.1°C. Recently, Roccato et al. (2017) analysed data on domestic refrigerator temperatures of chilled food in European countries in order to draw up general rules which could be used either in risk assessment or shelf life studies. In relation to domestic refrigerator temperatures, 15 studies provided pertinent data. Twelve studies presented normal distributions, according to the authors or from the data fitted into distributions. Analysis of temperature distributions suggested that the countries were separated into two groups: northern European countries and southern European countries. The overall variability of European domestic refrigerators in the latter study was described by a normal distribution: N (7.0, 2.7)°C for southern countries, and N (6.1, 2.8)°C for the northern countries.

Table 16: Temperature survey data on domestic refrigerators in the EU

Year reported	Country	N	Minimum temperature	Mean temperature	Max temperature	% refrigerators running at temperature °C[(a)]							Reference
						>4	>5	>6	>7	>8	>9	>10	
1990	UK	75		<5	15		6						(Rose et al., 1990)
1991	UK	252	0.9	6	11.4		70						(Evans et al., 1991)
1992	UK	150	0.8	6.5	12.6		71						(Flynn et al., 1992)
1993	France	102			14			70					(Victoria, 1993)
1994	Netherlands	125					70		28		2		(de Lezenne Coulander, 1994)
1997	Greece	136									50		(Sergelidis et al., 1997)
1997	UK	108	2	5.9	12		50						(Worsfold and Griffith, 1997)
1998	UK	645	-2	7	13		70						(Johnson et al., 1998)
2002	France	119	0.9	6.6	11.4		80						(Laguerre et al., 2002)
2003	UK	901					31					3	(Ghebrehewet and Stevenson, 2003)
2003	Greece	110				74		46		23		8	(Bakalis et al., 2003)
2005	Ireland	100	-7.9	5.4	20.7		59						(Kennedy et al., 2005a)
2005	Portugal	86						70					(Azevedo et al., 2005)
2005	Greece	258	-2	6.3				50				10	(Taoukis et al., 2005)
2005	Netherlands	31	3.8		11.5				68				(Terpstra et al., 2005)
2006	UK	24		5			33						(Breen et al., 2006)
2007	Spain	30		6.98[(b)]		83.7	74.0	61.9	48.5	35.3	23.6	14.5	(Carrasco et al., 2007)
2010	Greece	100	-0.3	6.3[(c)]	13.0	84	72	56	36	24	13	7	(Koutsoumanis et al., 2010)
2010	Spain	33	0.6	7.9	14.5	84.9		78.8		51.5		15.1	(Garrido et al., 2010a)
2010	UK	50		5.9			71			30	29		(WRAP, 2010)
2014	Italy	84	2.5	8.1	15.9	94			73.8			51.2	(Vegara et al., 2014)
2015	Sweden			5.9[(d)]						16			(Marklinder and Erikkson, 2015)
2016	UK	43	-1.7	5.9[(e)]	16.9	79.1	62.8	39.5	14.0	4.7	4.7	0.0	(Evans and Redmond, 2016a)

N: number of refrigerators sampled.
(a): Cumulative frequency of temperature data based on data reported by the authors.
(b): Estimated from fitted distribution of daytime data.
(c): Based on data from middle shelves.
(d): Based on data from middle shelves front.
(e): Central refrigerator temperature.

As in the case of retail cabinets temperature in domestic refrigerators varies among different positions. Figure 14 presents the temperature distribution (per cent) at the back and front of three different shelves (top, middle and bottom) in 1,812 refrigerators examined by Marklinder and Erikkson (2015).

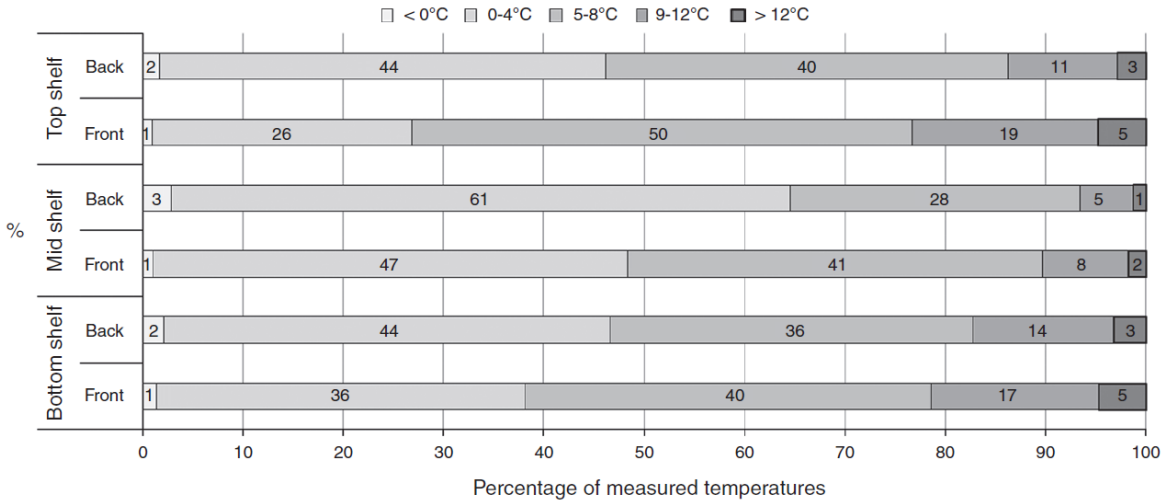


Figure 14: Temperature distribution (per cent) at the back and front of three different shelves (top, middle and bottom) in 1,812 refrigerators (adopted from Marklinder and Erikkson (2015) © Emerald Group Publishing Limited all rights reserved)

In general the middle shelf has been reported as the coldest spot of domestic refrigerators (Koutsoumanis et al., 2010; WRAP, 2010; Marklinder and Erikkson, 2015) and the door shelf as the hottest spot (Bakalis et al., 2003; Koutsoumanis et al., 2010).

Available data indicate that the domestic fridge mean temperature is also affected by fridge type and age. The waste and reduction action programme (WRAP, 2010) reported that fridge compartments at the bottom of the fridge–freezer combination showed slightly higher mean fridge air temperatures than both standalone (larder) fridges and fridge freezers with the fridge compartment on top. The results from the above programme also suggest a general trend that older fridges have higher mean air temperature than newer models. Fridges between one and two years old showed mean fridge temperatures of 3.7°C compared with mean fridge temperatures of 6.4°C within fridges over 5 years old.

3.3.4. Factors impacting the prevalence and concentration of *L. monocytogenes* in RTE food

Factors described in the EU-wide baseline survey

In the Scientific Report of EFSA (2014), the generalised estimating equations methodology was used to investigate the statistical association between several factors on which information was gathered during the BLS, and the two outcomes: prevalence of *L. monocytogenes* and proportion of samples with counts exceeding 100 CFU/g, in the surveyed fish and meat products. Problems due to sparseness of the data were evident during the model-building process and resulted in instability of the effect estimates of some factors during the sensitivity analysis. While some of the associations between the modelled outcomes and the examined factors were stable during sensitivity analysis, others were unstable with ORs and/or *p* values of the same factor fluctuating importantly between different analyses. One should be very careful with formulating strong statements about those factors that were unstable across different models during the sensitivity analysis. Therefore, the discussion of the respective results in the above-mentioned report, as well as in this section, focuses mainly on the factors which were significantly associated with the modelled outcomes, and exhibited consistent and stable associations in the presented models and the corresponding sensitivity analyses. Several other factors were included in the final multivariable models presented in EFSA (2014); however, the results were not always stable as shown in the sensitivity analysis, and therefore the results concerning these

factors are not presented here. In conclusion, the results presented in this section represent only a subsection of the terms included in the original multivariable models as they appear in the EFSA (2014) report and have also been statistically adjusted for all other terms that were included in those models. The complete models, as well as additional analyses, are presented in EFSA (2014) and in the External Scientific Report (Rakhmawati et al., 2014).

Based on the multivariable models for fish products, the odds of *L. monocytogenes* presence were higher for 'cold-smoked fish' than for 'hot-smoked fish' (OR = 0.54 and 0.61 at time of sampling and at end of shelf life, respectively) and 'unknown smoked fish' (OR = 0.57 and 0.62 at time of sampling and at end of shelf life, respectively), for 'sliced' than for 'not sliced' samples (OR = 1.59 and 1.39 at time of sampling and at end of shelf life, respectively) and for samples with 'two or more antimicrobial preservatives and/or acidity regulators (AP/AR),' than for samples with 'no reported AP/AR.' For this latter factor, the respective odds of *L. monocytogenes* presence were considerably higher (OR = 7.89 and 7.15 at time of sampling and at end of shelf life, respectively) in samples with two or more AP/AR than for samples with 'no reported AP/AR'. As discussed in EFSA (2014), initial appraisal of this association might appear as something of a paradox. However, most commercially used preservatives have a mild to moderate anti-listerial effect, which is essentially bacteriostatic (growth-inhibitory), rather than bactericidal. Hence, a higher number of preservatives in products contaminated with low numbers of *L. monocytogenes* could have only a minor and indirect effect on the probability of pathogen detection during food testing (a positive test). Antimicrobials may also reduce competitive flora possibly improving the growth potential of *L. monocytogenes*. Furthermore, any related conclusions or even attempts to interpret the association between the 'number of AP/AR' and *L. monocytogenes* prevalence should be made with great caution because the number of reported additives in this BLS does not necessarily constitute a reliable index of the anti-listerial 'load' or 'profile' of the fish products tested. In particular, the concentration of the reported additives was in most cases unknown and, additionally, food ingredients with direct or indirect antibacterial properties, e.g. salt, sugar, smoke or herbs (whose concentration was also, typically, unknown), were not taken into account in this analysis. Finally, some important explanations on how the sampled fish products were classified in the above-mentioned categories can be found in (EFSA, 2013). In conclusion, the reason for this finding is unknown and more studies are needed. Additionally, the models for the proportion of samples with counts exceeding 100 CFU/g, indicated that 'sliced' fish samples had higher odds of containing *L. monocytogenes* in excess of 100 CFU/g than 'not sliced' samples (OR = 2.79 and 2.55 at time of sampling and at end of shelf life, respectively).

As regards the factors associated with *L. monocytogenes* prevalence in the packaged heat-treated meat products, higher odds of *L. monocytogenes* presence were found for 'pâté' than for 'cold, cooked meat products' (OR = 2.91) and for 'sliced' samples than for 'not sliced' samples (OR = 2.13). The odds of being contaminated with *L. monocytogenes* for 'sausage' samples were not statistically significantly different from the corresponding odds for 'cold, cooked meat product' (OR = 0.97, *p* value = 0.93). For packaged heat-treated meat products, the proportion of samples with *L. monocytogenes* counts exceeding 100 CFU/g was associated with the 'animal species of the origin of the meat product' (lower odds, OR = 0.35 , for products made from meat from 'all other species' than for 'avian species') and with 'remaining shelf life' (the OR of having an *L. monocytogenes* count above 100 CFU/g was 1.01 for a meat product sample with an additional day of 'remaining shelf life' than for a sample with a 'remaining shelf life' that was one day shorter).

Finally, the association of *L. monocytogenes* prevalence and proportion of samples with counts exceeding 100 CFU/g with factors on which information was gathered during the BLS was not assessed for the surveyed cheese samples, owing to the very small number of samples that were found to be contaminated with *L. monocytogenes* in the BLS. More information can be found in EFSA (2014).

Factors described in the literature

The extensive literature search carried out by Jofré et al. (2016) on the *L. monocytogenes* contamination in different RTE foods considered risk factors associated with (a) the processing environment (e.g. presence/absence of HACCP systems, education and training of food handlers, validated cleaning and disinfection programmes, food contact surface testing/results), (b) manufacturing and preparation practices (e.g. type of processing, exposure after a lethal treatment, for instance during slicing and packaging, use of post-lethal treatment and/or antimicrobial process),

(c) product characteristics (e.g. pH, a_w, salt, preservatives, packaging type) and (d) storage conditions (e.g. time and temperature). The authors reported that the impact of some of the factors considered in the review was hard to assess, as the studies usually do not provide the outcome (prevalence and/or level values) as a function of the risk factors.

Only three studies were characterised as 'intervention studies,' because they were based on naturally contaminated samples for which there was reported prevalence of *L. monocytogenes* other than zero and because of that, the impact of different interventions on prevalence (also following storage) could be compared with that of a reference treatment. For example, the impact of super-chilling (-2ºC for 14 or 28 days) cold-smoked salmon before storage was assessed in comparison with a control (i.e. batch without super chilling) (Midelet-Bourdin et al., 2008). The prevalence of *L. monocytogenes* in smoked salmon super-chilled for 14 days was similar to the control (25 and 26%, respectively). Despite the fact that super-chilling for 28 days resulted in a slightly lower prevalence of the pathogen (23%) than the control, the number of samples with a concentration > 100 CFU/g was slightly higher than for the other treatments. Another study dealt with sources of contamination of *L. monocytogenes* in cold-smoked rainbow trout (Autio et al., 1999). An eradication programme consisting of disassembly and thorough cleaning and disinfection of the production machines and production lines caused a drastic reduction of *L. monocytogenes* prevalence in the environment and in the RTE product, e.g. from 100% (22 positives out of 22 analysed products) down to 0% (not detected in any of the 20 products analysed). More information can be found in Jofré et al. (2016). To conclude, there is a limited number of studies available dealing with interventions on naturally contaminated RTE foods.

3.3.5. Growth, survival and inactivation of *L. monocytogenes* in food and in the food chain

The previous EFSA Scientific Opinion about the risk of *L. monocytogenes* related to RTE foods (EFSA BIOHAZ Panel, 2008), reported on the predictive modelling tools and approaches that had become available before 2007, updating the opinion of 1999. The major advancements identified included the increase in availability of growth curves, the publication of new secondary models, the development of fitting tools and the incorporation of models to user-friendly applications. It reported on growth and probability of growth (growth/no-growth interface) models, but not on thermal and non-thermal inactivation. Since 2007 there has been an increasing volume of raw data published for growth and inactivation of *L. monocytogenes* in RTE foods, generated via challenge testing. This has enabled the improvement of existing models (e.g. by re-fitting), or the fitting of new models, as well as an increase in our understanding of the impact of factors influencing the behaviour of *L. monocytogenes* in RTE foods. The developments have also greatly assisted in quantifying the response of *L. monocytogenes* to spatio-temporal changes of the food processing and storage parameters (Augustin et al., 2015), including physico-chemical characteristics, structure and competing microflora. In the following paragraphs, the current Opinion provides an update on knowledge about growth and inactivation and the current state of the art of predictive modelling of *L. monocytogenes* in RTE foods since 2007, summarised in the following areas and further detailed in Appendix H. A detailed overview of the comparative impact of different models and modelling considerations on the estimated dose of *L. monocytogenes* may also be found in Pouillot and Lubran (2011):

- **Cardinal secondary** (describing how parameters of primary models such as maximum specific growth rates or lag times vary with environmental conditions) **growth and growth/no-growth models that predict the growth rate as well as the capacity of *L. monocytogenes* to initiate growth in response to multiple explanatory variables**. The basic idea behind cardinal parameter models (CPMs) is to use model parameters that have a biological and/or graphical interpretation and refer to minimum, optimal and maximum (or reference) values of product characteristics (intrinsic factors) and processing/storage conditions (extrinsic factors) that affect the growth of microorganisms. This has the advantage that appropriate starting values are easy to determine when models are fitted to experimental data by nonlinear regression. In addition, the models may be easily adjusted to account for different pathogen–food combinations by introducing the cardinal values and the maximum specific growth rate at optimum (μ_{opt}) or reference conditions (μ_{ref}) of the organisms in the target (e.g. new) food. They are also easily modified to account for an increasing number of factors influencing microbial growth, by simply adding multiplicative

gamma terms. The growth/no-growth interface divides the set of intrinsic and extrinsic factors controlling microbial growth into two domains, one where growth is permitted and one where growth is prohibited (Le Marc et al., 2005). It is delimited by the so-called cardinal values (T, pH, a_w, etc.) for growth and outlines the biokinetic range of microbial proliferation. Using the new form of CPMs with interactions (#4b in Table 46 of Appendix H), both the growth rate and the growth/no-growth interface of *L. monocytogenes* can be predicted simultaneously by identifying those combinations of growth factors (e.g. pH, a_w and T) that result in a psi value (ψ) equal to 1 or higher. A psi value equal to 1 defines the predicted growth/no-growth boundary; on the predicted no-growth side of the growth boundary, ϕ-values are higher than 1 and on the growth side they are lower than 1.

- **Strain variability and cardinal models with stochastic terms describing the strain variability in growth limits and growth rates**. In general, the growth variability among strains of *L. monocytogenes* appears to increase at growth conditions away from the optimum for this organism, or otherwise close to the growth boundaries (Barbosa et al., 1994; Begot et al., 1997; Lebert et al., 1998; De Jesus and Whiting, 2003; Lianou et al., 2006). For instance, the minimum inhibitory concentration (MIC) of *L. monocytogenes* to various organic acids has been shown to be strain- and pH-dependent, especially close to the growth-limiting pH (e.g. < 4.8), with the highest observed variation being almost 9.0 mM (Wemmenhove et al., 2016). The fact that cardinal (or growth-limiting) values are species- or even strain-dependent, introduces significant variability in the assessment of the impact of marginal growth conditions on microbial growth, a common issue encountered in quantitative microbiological risk assessment (Delignette-Muller and Rosso, 2000). Strain variability in growth limits can be incorporated into growth and growth/no-growth models by replacing the fixed values (commonly the median of reported cardinal values) for the cardinal parameters of intrinsic (e.g. pH, a_w and preservatives) and extrinsic (temperature, gas atmosphere, etc.) factors controlling growth of *L. monocytogenes* with probability distributions, thereby converting the deterministic models to stochastic ones (Ostergaard et al., 2015).

- **Impact of food microflora and food structure on the growth of *L. monocytogenes*.** This is about adding into the models a quantitative description of the additional complexity (and its impact on *L. monocytogenes*) of solid/semi-solid foods compared with broths or liquid foods, which have been the most common substrates for generation of modelling data. Growth of pseudomonads (e.g. in milk or meat) causes hydrolysis of proteins, which could provide free amino acids and likely stimulate *L. monocytogenes* growth (Marshall et al., 1992). Conversely, growth of *L. monocytogenes* is known to be negatively affected by the competitive growth of lactic acid bacteria, naturally present as indigenous (spoilage) microbiota or added as starter or aroma cultures in dairy products (Ostergaard et al., 2014). The proposed mathematical approaches to model the interaction between lactic acid bacteria and *L. monocytogenes* are mainly based on the Jameson effect model or the Lotka–Volterra competition model (Cornu et al., 2011), which consider that the growth of the pathogen starts to be affected (retarded or even halted but rarely stimulated) as the population of lactic acid bacteria (or of the competitor in general) approaches a critical level that is close to a stationary phase of growth. Such an approach has been successfully applied to model *L. monocytogenes* growth in processed seafood, mayonnaise-based seafood salads, pork products and cottage cheese, both at constant and fluctuating temperatures, deterministically and stochastically (Gimenez and Dalgaard, 2004; Cornu et al., 2011; Ostergaard et al., 2014; Mejlholm and Dalgaard, 2015).

Microbial growth in liquid laboratory media, in which most of the existing models have been developed, can differ significantly from growth on a solid food since in the latter the rates of diffusion of molecules are lower, the nutrients around a microcolony are utilised rapidly and not quickly replaced, while metabolites diffuse away slowly from the colony. If bacteria are suspended in liquids, their growth is planktonic and the motility of microorganisms may enable taxis to certain nutrient-rich sites of the food (Wilson et al., 2002). In structured aqueous media, due to the addition of thickeners, or structure-inducing agents, such as gelatin, pectins, starch, gums, etc., microbial cells are immobilised within the gelled regions and constrained to grow as submerged colonies in three dimensions. Their growth rates as colonies tend to be lower than that of planktonically growing cells (Wilson et al., 2002; Theys

et al., 2008; Aspridou et al., 2014; Boons et al., 2014; Skandamis and Jeanson, 2015). This can be further enhanced by increasing the fat concentration on the expense of water phase, thereby increasing the size of oil droplets. If bacteria are growing on the surface of foods, such as meat and vegetables, growth is also colonial, initially in two dimensions (mono-layer), whereas the centre of the colony gradually develops in the third dimension, most likely upward, depending on aeration and nutrient availability (Skandamis and Jeanson, 2015).

- **Impact of pre-culture conditions and shifts in the food (micro-) environment on the lag time of *L. monocytogenes***, also addressing the impact of **innate single cell heterogeneity of lag times** on overall population dynamics. The number of models for the growth rate of *L. monocytogenes* is markedly higher than that for lag time. Lag time depends on current growth conditions and on cell 'history,' which defines the capacity of the organism to adapt and re-grow in the new environment. Studies have demonstrated the effect of pre-incubation conditions (composition of the medium, temperature, pH, a_w, etc.) on the lag duration of different pathogens and recent reports quantitatively describe the impact of up- and downshifts in salinity and pH on the lag time of *L. monocytogenes* (Le Marc et al., 2010; Belessi et al., 2011b). It is suggested that there is an adaptation or injury rate induced at conditions inhibiting the growth of *L. monocytogenes* (Belessi et al., 2011b). Another situation that may strongly impact the physiological state of cells is their life within a biofilm. Detachment of such cells from the biofilm and translocation to a food (e.g. due to contamination) may be sensed as a shift in the environment and thus induce lag time (Poimenidou et al., 2009; Belessi et al., 2011a).

Traditional predictive microbiology uses deterministic mathematical models which describe the growth of large microbial populations as a whole without considering the variability in the responses of individual cells. Since contamination with pathogens usually occurs with very low numbers, the development of stochastic approaches that can describe the variability of single cell behaviour is necessary for realistic estimations of safety risks. Koutsoumanis and Lianou (2013) showed that as a result of the heterogeneity in cell division time, growth of single cells or small microbial populations present a high variability, and can be considered as a pool of events, each one of which has its own probability of occurring. In addition, the apparent variability in population growth gradually decreases as the initial population increases (i.e. at time 0). A significant heterogeneity has also been observed in the ability of individual cells to initiate growth (Aguirre and Koutsoumanis, 2016).

- **Thermal and non-thermal inactivation models**. Fewer inactivation models than growth models have been reported and in Table 50 (Appendix H), an overview of the available inactivation models for *L. monocytogenes* is provided. Non-thermal inactivation is usually the result of the single or combined effect of low pH (< 4.5) or a_w (< 0.90) and moisture (< 60%) at refrigeration or ambient temperatures in the presence or not of preservative agents close to their MIC. Although the lethality is attributed to heat-independent factors, temperature values within the biokinetic range of growth from the minimum (suboptimal: 0–5°C) to the maximum (super-optimal: 45–47°C) value for growth, remain the factor governing the non-thermal inactivation rate of bacteria (Shadbolt et al., 1999; Ross et al., 2008; McQuestin et al., 2009; Zhang et al., 2010a). The work of Coroller et al. (2012) presents a modelling approach for non-thermal inactivation based on the gamma hypothesis, capable of quantifying both growth and inactivation depending on the prevailing conditions.

3.3.6. Summarising remarks for exposure assessment

- Persistence of *L. monocytogenes* in food processing environments is an often observed and important phenomenon for contamination of RTE foods. Some hypovirulent molecular subtypes such as ST 121 seem to encompass multiple isolates with a proven capability to persist.

- Whether persistence is a result of improper hygiene conditions or more the effect of strains equipped with an arsenal of genetic determinants is under debate. A high adaptive capacity against physical–chemical factors and biofilm-forming capacity could partly explain the persistence phenomenon. A transposon (Tn6188) and the bcrABC cassette were shown to be associated with tolerance against some disinfectants. The hypervariable genetic hotspot

lmo0443-lmo0449 appears to play a further role in stress response as it may harbour two independently acting stress survival islets (either SSI-1 or SSI-2).

- During the time period 2008–2015, non-compliance at processing ranged from 3.5 to 9.6% for 'RTE fishery products,' from 0.9 to 6.8% for 'RTE products of meat origin other than fermented sausage,' and from 0 to 0.6% for 'RTE products of meat origin, fermented sausage.' Non-compliance ranged from 0.2 to 1.8% for 'soft and semi-soft cheese' and 0 to 0.3% for 'hard cheese.' At retail, non-compliance was generally lower and for most of the years was less than 1%. The lower level of non-compliance at retail is at least partly explained by the application of the different limits of FSCs for retail and processing.
- The extensive literature survey (outsourcing activity 1) covering the time period 1990–2015 reported that the distribution of the *L. monocytogenes* prevalence values was asymmetric, with several outliers and extreme values. For the whole period, the median of the prevalence was below 10% for all subcategories, except for fermented sausages (10%), cold-smoked fish (13%), smoked fish (either cold- or hot-smoked; 12%) and cured/salted fish (12%). There was wide variability between studies due to, for example, the aim of the study, the foods sampled and the geographical origin.
- According to the EU-wide BLS conducted in 2010 and 2011 on *L. monocytogenes* in three RTE food categories at retail:
  - at the end of shelf life *L. monocytogenes* was more prevalent in RTE smoked and gravad fish (10.3%, and 1.7% above 100 CFU/g), than in RTE heat-treated meat (2.1%, and 0.43% above 100 CFU/g) and RTE soft and semi-soft cheese (0.5% and 0.1% above 100 CFU/g) products.
  - Based on the multivariable models, the odds of *L. monocytogenes* presence in sliced sampled fish and meat RTE products were higher than in non-sliced products.
  - Additionally, the odds of *L. monocytogenes* presence were higher for 'cold-smoked fish,' than for 'hot-smoked fish' and 'unknown smoked' fish. Moreover, the odds of *L. monocytogenes* presence were considerably higher for fish samples with two or more AP/AR than for samples with 'no reported AP/AR'. The reasons are unknown and more studies are needed.
  - Of factors associated with prevalence of *L. monocytogenes* in packaged heat-treated meat products, higher odds of presence were associated with 'pâté' than with 'cold, cooked meat products,' whereas the odds were not significantly different for `sausage´. Similarly, the proportion of samples with *L. monocytogenes* counts exceeding 100 CFU/g was associated with the 'animal species of the origin of the meat product' and with 'remaining shelf life'.
- The average *L. monocytogenes* concentration found among RASFF notifications related to RTE foods was 2.61, 2.46 and 2.34 $\log_{10}$ CFU/g for the categories 'milk and milk products,' 'fish and fish products' and 'meat and meat products other than poultry,' respectively. The respective highest maximum concentrations were reported as 6.25 $\log_{10}$ CFU/g, 5.32 $\log_{10}$ CFU/g, and 4.75 $\log_{10}$ CFU/g. The concentration was over 2 $\log_{10}$ CFU/g in approximately 80% ('fish and fish products') and 65% ('milk and milk products,' 'and 'meat and meat products other than poultry') of notifications.
- Cooked meat and heat-treated sausage were the subcategories with most consumed servings per person and year in the EU/EEA and for meat products the number of servings was in general greater for males than for females.
- A combination of results from the BLS and consumption data indicates that approximately 55 million servings contaminated with more than 100 CFU/g may be consumed by the ≥ 75 age group per year in the EU/EEA.
- Unsafe practices (including storage time and temperatures) are not uncommon within the elderly group (> 10% of persons studied), and can have a potential impact on the human listeriosis risk. There is a wide variation within broadly defined consumer groups and it is thus

problematic to generalise about food handling behaviours of these groups and on how this may contribute to trends of human listeriosis.

- The extent of different behaviours among risk groups may vary between Member States to the same extent that socioeconomic factors, traditions and types of food vary. Since the majority of studies on food handling are from a few countries only this may lead to some uncertainty about the generalisability of the results presented.
- Temperature of domestic refrigerators is highly variable. A review of 23 available survey studies from 1991 to 2016 showed mean, minimum and maximum temperatures ranging from < 5 to 8.1, -7.9 to 3.8 and 11.4 to 20.7, respectively. A recent analysis of domestic refrigerator temperature distributions suggested that the countries were separated into two groups: northern European countries (normal distribution: N (6.1, 2.8)) and southern European countries (normal distribution: N (7.0, 2.7)).
- Developments with cardinal growth, and probability of growth models as well as non-thermal inactivation models are promising and have improved the capability to provide realistic predictions for growth initiation and changes in levels of *L. monocytogenes* in RTE foods.
- Knowledge gaps make it difficult to draw quantitative conclusions on the range of food handling behaviours in different risk and age groups and on how this may contribute to trends of human listeriosis. In this context, there is a need for better information on human listeriosis cases in terms of socioeconomic–demographic data.
- There is a need to better understand how the dietary practices and food handling of the elderly are affected by ageing and how this may be linked to an increased exposure to *L. monocytogenes*.
- To improve the performance of cardinal models there is a need to better understand the lag time and the adaptive responses to environmental shifts both at single cell and population level, as well as the quantitative impact of intrinsic factors (e.g. food structure, indigenous microflora) on the growth and survival of *L. monocytogenes*.

3.4. Evidence for risk characterisation – summary of recent risk assessment studies

3.4.1. Results from the review of QMRA outputs

The available QMRA studies from the literature were retrieved by Pérez-Rodríguez et al. (2017) and reviewed. These studies performed quantitative risk assessment and covered deli meats sliced at retail or pre-packaged, vacuum or non-vacuum packaged, soft cheeses made of both pasteurised and non-pasteurised milk, smoked fish including gravad salmon, rainbow trout, pasteurised milk and fresh produce (leafy vegetables). Regarding the approach to address variability and uncertainty, both first and second order approaches were undertaken. The influential factors on risk estimate included: (i) time and temperature at different stages of the food chain, mainly during distribution and storage at retail or at consumer level, (ii) the food's intrinsic characteristics (e.g. pH, a_w, presence of inhibitors), (iii) extrinsic factors (e.g. packaging atmosphere), (iv) application of lethality treatment such as heat treatment (pasteurisation), (v) likelihood of transfer due to slicing or other handling steps, such as partitioning or cross-contamination by the processing environment, (vi) prevalence and concentration of *L. monocytogenes*, (vii) susceptibility of population, (viii) serving size and (ix) number of servings.

Assessment of the impact of the aforementioned factors on the final risk estimate was done either through sensitivity analysis, estimating the correlation coefficient between the model inputs (for exposure assessment and dose response) with the outputs of concern, e.g. mean number of human listeriosis cases per year, or via 'what if' scenario analysis and importance analysis, in relation to a baseline scenario, or a combination of both. Some studies performed detailed ('advanced') sensitivity analysis by assigning values for certain input parameters at specified percentiles (in the range of 1 to 99%) of their distribution, leaving the other model input parameters to vary according to their own distribution and performing Monte Carlo simulations to estimate the change in model output (e.g. number of cases) as a result of input shifts in the specified percentiles (Carrasco et al., 2010; Mataragas et al., 2010; Pradhan et al., 2010; Pradhan et al., 2011; Stasiewicz et al., 2014).

Assessment of risk was based on the following three groups of population: perinatal (fetuses and newborns from 16 weeks after fertilisation to 30 days after birth), the elderly population (> 60 or > 65 years old) and intermediate population that does not belong to either of these categories. When a single population group was considered in the risk characterisation, then either the elderly, or the perinatal subpopulations, or collectively the high-risk fraction of a national population was used. In some cases (e.g. see Carrasco et al. (2010)) the risk estimates for the high-risk population were compared with those of the low-risk population. Expressions of risk included cases per year or per serving.

According to the sensitivity or scenario analysis of the QMRA studies reviewed, the factors per food category identified as being influential on the risk of human listeriosis per serving or per year have been summarised below.

Deli meats

A risk assessment for *L. monocytogenes* in deli meats predicted that 63–84% of human listeriosis cases and deaths attributable to deli meats are due to retail-sliced products (Gombas et al., 2003; FSIS, 2010; Pradhan et al., 2011). Sensitivity and scenario analyses performed by Pradhan et al. (2011) indicated that the frequency of cross-contamination at retail from other food products or from the environment was the most important factor that affected the relative risk of listeriosis-associated deaths. It was estimated that cross-contamination of deli ham and turkey from other products increased the relative risk of listeriosis-associated deaths 5.9- and 6.1-fold, respectively, and from the retail environment 4.9- and 5.8-fold, respectively.

The prevalence and levels of *L. monocytogenes* at the processing plant, the stage of product slicing, storage time and temperature at retail and at the consumer level, as well as the presence of growth inhibitors are affecting the risk of listeriosis (Endrikat et al., 2010; Garrido et al., 2010b; Gallagher et al., 2013). Retail-sliced products represent a 2- to 4-fold higher risk (also expressed through the number of deaths) than pre-packaged sliced products (Endrikat et al., 2010; Gallagher et al., 2013). Endrikat et al. (2010) carried out a risk assessment of pre-packaged RTE meat and poultry foods produced by federally inspected processing facilities in the period from the early 1990s to 2008. Notably, the decreasing trend in the prevalence of *L. monocytogenes* at production (and the expected concomitant decline in the human listeriosis incidence rate) was counteracted by the increase in prevalence at retail due to slicing. It was suggested that this resulted in the constant listeriosis rates observed from 2001 onwards (Endrikat et al., 2010).

The elevated risk posed by products sliced at retail is reduced almost 2.8- to 9-fold if growth inhibitors are used in the formulation of the cooked meat products (Pradhan et al., 2010). According to the modified version of the 2003 FDA and FSIS (FDA and FSIS, 2003) model for the assessment of the relative risk of *L. monocytogenes* in 23 categories of RTE products, almost 70% of the estimated deaths caused by consumption of contaminated deli meats were attributed to retail-sliced products that did not contain growth inhibitors (Endrikat et al., 2010; FSIS, 2010). The prevalence and levels of *L. monocytogenes* on products when they leave the plant in combination with their ability to support growth of the organism are influential factors on consumers' exposure (Garrido et al., 2010a; Gallagher et al., 2013).

Storage temperature has a higher influence on the risk of listeriosis than storage duration. Reduction of the home storage temperature of vacuum-packaged cooked ham and turkey to below 7°C confers a marked reduction in the risk. Nonetheless, long storage time at retail (i.e. longer shelf life) or at home only, especially in combination with improper temperature control, may significantly increase the risk (Gallagher et al., 2013).

The sensitivity analysis of Mataragas et al. (2010) for cooked meat products, targeting the high-risk fraction of the EU population (approx. 20–25%), suggests that a retail storage temperature of 7°C or home storage temperature of 9.4°C, a storage duration of 22 d at retail and 5 d at home are the cut-off values for a steep increase in the risk of listeriosis. The use of antimicrobials in the formulation or the application of post-lethality antimicrobial interventions is thought to contribute to further risk reduction.

Cross-contamination from the retail environment, e.g. due to slicing, or other contaminated products increases the risk more than the increase in the prevalence of *L. monocytogenes* in the unopened

products (Pradhan et al., 2011). Controlling cross-contamination, e.g. by GHP, the use and frequent replacement of gloves, equipment sanitation, elimination of niches and early slicing, contributes to the control of the risk. Serving size is thought to have the least effect on the risk.

Fish products

The dominant factors that seem to affect the risk estimate in this product category are those determining the concentration of the pathogen at the time of consumption (Lindqvist and Westoo, 2000; Pouillot et al., 2007; Pouillot et al., 2009). As such, control of temperature (< 4.5°C) throughout the supply chain in combination with short storage periods (e.g. < 7 d in home refrigerators) and compliance with microbiological criteria, i.e. < 100 CFU/g, at the moment of food purchase, especially in the trout model, were identified as the most effective means for controlling the risk of listeriosis (Garrido et al., 2010b). According to Pouillot et al. (2007) and Pouillot et al. (2009) employing second order exposure assessment and risk characterisation models, respectively, listeriosis caused by consumption of cold-smoked salmon is attributable to the rare consumption of products with high doses, due to growth of *L. monocytogenes*. The number of listeriosis cases correlated well with the frequency of exposure to 10^8 CFU/serving (Pouillot et al., 2009). As such, the mitigation strategies that may reduce the risk of listeriosis in this product are those associated with the reduction of prevalence and actions taken at the consumer phase, such as limiting the temperature abuse (e.g. by lowering the mean temperature in domestic refrigerators by 2–3°C) and reducing the duration of domestic storage, i.e. from purchase to consumption. In contrast, the control of initial contamination is less effective in reducing the risk, unless the growth of *L. monocytogenes* is sufficiently controlled post-packaging up to the moment of consumption (Pouillot et al., 2009).

In the same context, other factors to which the final risk estimate was found to be sensitive were the growth rate of lactic acid bacteria acting as competitors to *L. monocytogenes*, the variability and the uncertainty around the mean parameter of the reference growth rate of *L. monocytogenes*, the variability in the minimum growth temperature of *L. monocytogenes*, and the variability in consumer refrigerators and in the proportion of consumers exposed to contaminated products (Pouillot et al., 2007; Pouillot et al., 2009; Vasquez et al., 2014).

The predicted risk is also highly affected by the DR model used and it is important that the model sufficiently represents the virulence properties (also in relation to human susceptibility) of various *L. monocytogenes* strains (Lindqvist and Westoo, 2000). In the Pouillot et al. (2009) model, the uncertainty in the *r* parameter of the DR model, representing the probability of infection per single cell, was the major influential factor of the uncertainty in the predicted number of listeriosis cases.

Dairy products

For raw milk, first the storage temperature and then the time between collection of milk in the bulk tank and the purchase are the most critical factors affecting the risk of listeriosis. The longer the time the higher the risk (Latorre et al., 2011). Purchasing raw milk from retail stores leaves more time for *L. monocytogenes* to grow and thus increases the risk. According to Latorre et al. (2011), existence of microbiological raw milk testing programmes may contribute highly to risk reduction. The susceptibility of different consumer groups was assessed with the following decreasing order: elderly > perinatal > intermediate.

Increasing the milk pasteurisation temperature from 72 to 82°C, in the context of high temperature short time processing, may be associated with an increase of human health risk from listeriosis. This is possible because in the event of post-process contamination of milk with *L. monocytogenes*, the organism may grow faster and at higher maximum levels than in milk pasteurised at a lower temperature. This is due to the lower levels of competing microbiota achieved by increasing the pasteurisation temperature (Stasiewicz et al., 2014). The rise in risk is further supported by improper consumer practices in the storage of milk, i.e. increases in temperature and storage duration. By contrast, boiling milk in vending machines markedly reduces the risk of listeriosis (Giacometti et al., 2015).

In Canada and the USA, it was estimated that raw milk cheeses pose a 53 and 112 times higher risk, respectively, than cheese made from pasteurised milk. Lethality treatments may reduce the risk, but only 100% testing of lots and removing the positive ones may ensure higher risk reduction than that

ensured by using pasteurised milk for cheese manufacturing (Williams et al., 2009; FDA and Health Canada, 2015).

In soft cheeses made from raw milk, such as camembert and brie, growth is expected to be higher in the rind than in the core, but the overall risk seems to be low and controlled by the competitive growth of curd acidification by starters (Sanaa et al., 2004). The final risk estimate is sensitive to the speed and strength of curd acidification.

In soft-ripened cheese made of pasteurised milk, the factors impacting the risk of illness by *L. monocytogenes* are cross-contamination or re-contamination during manufacturing (linked to the hygiene of the processing environment), the control of *L. monocytogenes* concentration in cheese entering the ripening room and the time–temperature and the ageing of the cheese at retail (Tenenhaus-Aziza et al., 2014).

Leafy vegetables

Storage temperature at retail and in the home, duration of storage and serving size are the most influential factors for the risk of listeriosis derived from leafy greens intended to be eaten raw (Tromp et al., 2010; Ding et al., 2013; Sant'Ana et al., 2014). In salad bars, the amount of products in stock and turnover times may further contribute to risk since this will influence the probability of having products out of date or of lower quality (Franz et al., 2010; Tromp et al., 2010).

3.4.2. Results from the outsourcing activity 2 risk assessment

A quantitative risk characterisation of *L. monocytogenes* in various RTE food categories (heat-treated meat; smoked and gravad fish; and soft and semi-soft cheese) in the EU was performed, starting from the retail stage. In principle, the three major RTE food categories (meat, fish and dairy) that were considered by the BLS in 2010 and 2011 were considered. The three categories were divided into seven subcategories, including cooked meat, sausage and pâté, cold- or hot-smoked fish and gravad fish and soft/semi-soft cheese.

For prevalence and concentration, data from the BLS were complemented with EU monitoring data and data from other sources: (i) the BLS data, (ii) the EU monitoring data (2011–2014) and (iii) scientific studies retrieved by Jofré et al. (2016).

For modelling purposes, prevalence of *L. monocytogenes* in the major RTE food subcategories was considered for further splitting into food subcategories, based on their relevance to risk and according to the review analysis. In particular, prevalence scenarios were considered for sliced/non-sliced RTE foods as well as for the type of atmosphere packaging, i.e. reduced oxygen packaging (ROP) and normal.

Input data were introduced as distributions into the stochastic risk assessment model and different scenarios and sensitivity analyses were carried out. Growth of *L. monocytogenes* considering interaction with lactic acid bacteria was modelled from retail to consumption using temperature–time profiles during food transport and storage. This information was combined with the Pouillot et al. (2015) DR model (see Section 3.2.3.) to estimate the number of listeriosis cases per million servings (reflecting individual and food-related risk; Appendix I) and per year (reflecting population risk or public health burden, also associated with consumption frequency; Table 17) in the EU separately for the 'healthy' population (< 65 years), the elderly (≥ 65 years) and pregnant women by varying the parameter of the DR model for the different risk groups.

Heat-treated meat products

For heat-treated meat products, results showed that the type of product exerted a noticeable effect on the incidence rates of human listeriosis. According to the simulation outcome, pâté presented the highest listeriosis risk (2.14×10^{-5} – 2.51 cases/10^6 servings) followed by cooked meat (2.72×10^{-4} – 1.26 cases/10^6 servings) and sausage (1.96×10^{-5} – 8.28×10^{-1} cases/10^6 servings) (Appendix I). In pâté, the population group with the largest risk per million servings was pregnant women, followed by the elderly and finally the healthy population. The package atmosphere and slicing appeared to affect listeriosis risk across all population groups, and this was most evident in the pregnant population. In all cases, ROP and slicing led to the lowest listeriosis risk. In contrast, the combination with the

greatest total risk values corresponded to normal atmosphere packaging and slicing, indicating that slicing becomes a relevant factor contributing to listeriosis risk when combined with normal packaging. Predicted risk levels associated with cooked meat were highest for the pregnant population. Sausage products presented a lower risk than cooked meat and pâté.

According to Pérez-Rodríguez et al. (2017) the uncertainty range in the estimates is important to consider when types of product and populations are compared. However, the reported 95% CI reflects mostly variability not uncertainty since only the uncertainty of the prevalence estimate was considered. In some cases, the 95% CI ranged more than one order of magnitude (e.g. in sausage with normal and non-slicing conditions for the elderly), indicating that those risk estimates are associated with a large variability and should be carefully interpreted.

As regards the type of product, i.e. type of package atmosphere and slicing/non-slicing, a definitive and general conclusion was not drawn by Pérez-Rodríguez et al. (2017). Overall, it appears that slicing and normal atmosphere are more often related to a higher listeriosis risk, although this was not a general rule and, for example, for pâté, the highest combination corresponded to sliced and ROP packaged pâté. It is likely that the combined effect of prevalence and the shelf life associated with each product could play a relevant role in these differences.

Smoked and gravad fish

Concerning the predicted number of cases per million servings, the median value is, in general, higher in cold-smoked fish or gravad fish depending on conditions, and lower in hot-smoked fish (Appendix I).

The predicted number of listeriosis cases per million servings was similar for sliced and non-sliced products under both ROP and normal atmosphere packaging.

For hot-smoked fish, the model predicted a much lower number of cases (10 times lower) than for cold-smoked fish due to the lower prevalence values, and the expected lower growth rate of *L. monocytogenes* during storage. The population group exposed to the largest risk was again pregnant women, followed by the elderly and finally the healthy population. The ROP/sliced condition was associated with the highest predicted number of listeriosis cases.

The scenario associated with the highest risk was that corresponding to exposure of the pregnant population to gravad fish sliced and packed with normal atmosphere packaging or ROP. For this scenario, a median of 1.1 cases/10^6 servings is predicted, with a 2.5% and 97.5% percentile of 0.7 and 1.6 cases/10^6 servings, respectively. As for cooked meat, risk estimates for the pregnant population were associated with a large variability and results should be carefully interpreted. For the elderly and healthy subpopulation, predicted risk for the RTE fish category was in general 10 and 100 times lower than for the pregnant population, respectively.

Soft and semi-soft cheese

Predicted risk per million servings for soft and semi-soft cheese indicates substantial effects from the slicing procedure. Specifically, slicing doubled the predicted risk associated with soft and semi-soft cheese independent of population group. For instance, for the pregnant population the median number of predicted cases per million servings was 1.07×10^{-3} for non-sliced and 1.98×10^{-3} for sliced cheese. The predicted risk for the pregnant population group was 100 times greater, expressed as listeriosis cases per million servings, than for the elderly population.

In the risk model, predicted growth was low for all evaluated scenarios independent of packaging atmosphere. Moreover, for this RTE food category, it seems that the effect of slicing contributed mostly to the increase in the number of cases due to an increase in prevalence.

Predicted number of total cases – public health burden

The overall risk estimates were obtained for each food subcategory expressed as listeriosis cases per year in the EU, derived from the number of servings in the EU Member States and their proportions on the market (Table 17). The model estimated 2,318 (95^{th} percentile interval: 1,450–3,612) listeriosis cases per year in the EU considering the seven RTE food subcategories altogether. The overall results indicated that the higher risk population group was the elderly population to which 48% of total cases

were attributed, followed by pregnant women (41%), and finally the < 65-year-old population (11%) (see Table 18).

The results per food category showed differences in this aspect. For pâté, sausage, soft and semi-soft cheese and cold-smoked and gravad fish most cases were attributed to the elderly population and for cooked meat and hot-smoked fish to the pregnant population. The product that obtained the highest median number of predicted listeriosis cases was cooked meat, closely followed by sausage.

Regarding smoked and gravad fish, the highest number of listeriosis cases was predicted for the subcategory gravad fish in the elderly subpopulation (median = 230 cases), followed by cold-smoked fish in the elderly population (median = 201 cases) and the pregnant population (median = 104 cases). In general, the elderly subpopulation was predicted to be by far the most affected, especially when consuming gravad fish. However, for other food subcategories, such as hot-smoked fish, only a slight difference in the number of cases between the three subpopulations (six cases for the pregnant women, one case for elderly women, and no cases for the healthy subpopulation) was predicted.

Finally, for soft and semi-soft cheese, the elderly population was associated with the highest number of predicted cases (median = 11) followed by the healthy and pregnant population groups.

Uncertainty and sensitivity analysis

Pérez-Rodríguez et al. (2017) identified and described sources of uncertainty in the risk assessment model. For the assessment of uncertainty, the effect of individual variables was qualitatively assessed by determining each one's direction on the increase or reduction in the final number of human listeriosis cases per year in the EU population. A quantitative assessment of uncertainty was not done except for the uncertainty in the prevalence estimate. This was stated to be due to the scarcity of data and information for some variables. Thus, the combined effect of all uncertainties was not quantified.

To determine the influence of the most important model inputs on the estimated number of human listeriosis cases, Pérez-Rodríguez et al. (2017) performed scenario analyses with a focus on those variables deemed to be important sources of uncertainty in the model. The selected variables were modified to values representing worst and best case scenarios. Results were expressed as variation percentages (%) in the number of cases with respect to the outcome from the baseline model expressed per million servings to enable comparisons between food categories with different consumption patterns.

Different factors had different impacts on estimated risk in the different food categories. In general, the most important factor was storage temperature and the effect was greatest for heat-treated meat. The assumption on the maximum concentration of *L. monocytogenes* in a serving impacted on the estimated risk for all food categories but especially for RTE fish. The effect of the time to consumption was fairly small except for RTE fish. The assumption of the presence of a lag time or not was also small for all food categories and introducing lag time only reduced the estimated risk in heat-treated meat not RTE fish or RTE cheese.

Table 17: Estimation of the number of human listeriosis cases per year in the EU in the ready-to-eat (RTE) food subcategories (adopted from Pérez-Rodríguez et al. (2017))

RTE food subcategory	Population subgroups			
	Healthy[(a)]	Elderly[(b)]	Pregnant	Total
Cold-smoked fish	54 (42, 68)	201 (154, 254)	104 (75, 138)	358 (271, 460)
Hot-smoked fish	NC (NC, 1)	1 (NC, 1)	6 (4, 8)	7 (4, 10)
Gravad fish	48 (33, 70)	230 (160, 320)	92 (63, 129)	370 (257, 519)
Cooked meat	71 (50, 98)	316 (218, 449)	477 (337, 659)	863 (604, 1207)
Sausage	64 (31, 118)	252 (120, 469)	225 (107, 417)	541 (258, 1003)
Pâté	12 (4, 27)	92 (28, 220)	54 (16, 130)	158 (48, 377)
Soft and semi-soft cheese	5 (2, 10)	11 (5, 20)	3 (1, 6)	19 (8, 36)
Total	254 (162, 392)	1,103 (685, 1,733)	961 (603, 1,487)	2,318 (1,450, 3,612)

Numbers outside brackets represent 50th percentile. Numbers between brackets represent 2.5 and 97.5th percentiles. NC stands for no cases, which refers to values < 0.5 cases/year; for values between 0.5 and 1, the values have been rounded to 1. Total refers to the arithmetic sum of the number of cases.

(a): < 65 years old.

(b): ≥ 65 years old.

Table 18: Attribution of the yearly estimated 2,318 human listeriosis cases in the EU to the population subgroups and the ready-to-eat (RTE) food subcategories (derived from Table 17)

RTE food subcategory	Population subgroups			
	Healthy[(a)]	Elderly[(b)]	Pregnant	Total
Cold-smoked fish	2.3	8.7	4.5	15.5
Hot-smoked fish	0.0	0.0	0.3	0.3
Gravad fish	2.1	9.9	4.0	16.0
Cooked meat	3.1	13.6	20.6	37.3
Sausage	2.8	10.9	9.7	23.4
Pâté	0.5	4.0	2.3	6.8
Soft and semi-soft cheese	0.2	0.5	0.1	0.8
Total	11.0	47.6	41.4	**100.0**

(a): < 65 years old.

(b): ≥ 65 years old.

3.4.3. Summarising remarks for risk characterisation

- Most risk characterisations consider three risk populations (i.e. pregnant women/perinatals (fetuses and newborns from 16 weeks after fertilisation to 30 days after birth), the elderly (> 60 or > 65 years old), and the intermediate population that do not belong to either of these categories) and have not addressed gender differences. This limitation can be addressed with dose–response (DR) data and other input data developed at a finer resolution in some recent publications and in the present Opinion.
- The importance of growth as a risk determining step is reinforced in the review of published risk assessments and levels of important factors reducing growth (such as storage times, storage temperatures, antimicrobials, competition) have been reported under different assumptions and scenarios.
- At retail, cross-contamination from other products and from the retail environment (including during slicing) to RTE foods has been identified as important for the predicted risk of human listeriosis.
- Based on the quantitative risk characterisation of *L. monocytogenes* in various RTE food categories (heat-treated meat; smoked and gravad fish; and soft and semi-soft cheese) in the EU:
  - The food subcategory associated with the largest number of cases per year was cooked meat (863 cases). After that followed sausage (541 cases), gravad fish (370 cases), cold-smoked fish (358 cases), pâté (158 cases), soft and semi-soft cheese (19 cases) and hot-smoked fish (7 cases). For hot-smoked fish and for cooked meat most of these cases were attributed to the pregnant population, for the rest of the food subcategories most cases were attributed to the elderly population (≥ 65 years old). The fewest cases were attributed to the healthy population (< 65) for all food categories.
  - Estimated risks expressed as the median number of cases per million servings was in general highest for the pregnant population, followed by the elderly and last the healthy population.
  - Similarly, the estimated median number of cases per million servings for RTE meat for all scenarios and populations ordered by the range was pâté (2.14×10^{-5} – 2.5 cases) followed by cooked meat (2.72×10^{-4} – 1.26 cases) and sausage (1.96×10^{-5} – 8.28×10^{-1} cases). For RTE fish, gravad fish (2.16×10^{-3} – 1.57 cases), cold-smoked fish (3.02×10^{-4} – 2.34×10^{-1} cases), and hot-smoked fish (4.94×10^{-7} – 4.55×10^{-4} cases), and for soft and semi-soft cheese (4.39×10^{-6} – 1.95×10^{-2} cases).
  - Most of the cases were predicted in the elderly population (48%) followed by the pregnant population (41% of cases) and the healthy population (11%).
  - Uncertainty sources for some variables such as initial prevalence should be further elucidated as well as variability in *L. monocytogenes* growth when types of product and populations are compared.
  - The evaluated input variables had different impacts on estimated risk in the different food categories. In general, the most important factor was storage temperature, and the effect was greatest for heat-treated meat.
  - The assumption on the maximum concentration of *L. monocytogenes* in a serving had an impact on the estimated risk for all food categories but especially for RTE fish. The effect of the time to consumption was fairly small except for RTE fish. The assumption of the presence on lag time was also small for all food categories.

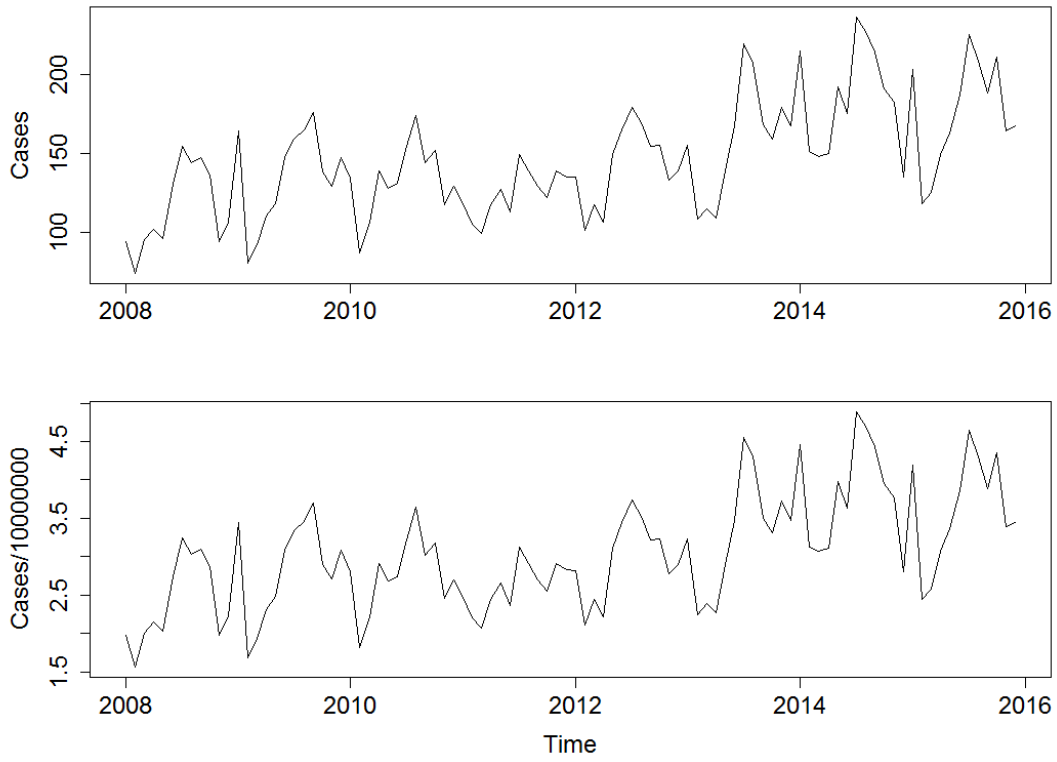
3.5. Evaluation of the epidemiological trend of human listeriosis

3.5.1. Results of the aggregated TSA

In this section, the analysis of the aggregate *L. monocytogenes* series from January 2008–December 2015 is presented. This analysis modelled confirmed human listeriosis cases per month. This was also the outcome used in the analysis described in Section 1.1. (EFSA and ECDC, 2015) that led to the conclusion of an increasing trend. Furthermore, the underlying total population (denominator) did not change meaningfully and the analysis of the incidence rates would give the same results. This is in contrast with an analysis by age–gender subgroups, in which the variation may be more significant. As mentioned in Section 2.2.1, the 2008–2015 time series exhibits changing dynamics and requires a dynamic linear modelling approach. A random walk model with seasonal effects (Equations 1 and 2) and a local linear growth model (i.e. a second order trend model) (Equations 3 and 4) were finally selected as appropriate to model these data.

For the simple random walk plus seasonal trend model the maximum likelihood estimates of the variances for the two equations are $V = 29.9$ and $W = 184.3$. This means that most of the variation (more than six times more) of the *L. monocytogenes* series is explained by the random walk and the seasonal patterns in the data. With the selected model, the total variance of the time series is partitioned as a random walk and seasonal model across two equations. This means that about 86% (= 184.3/(184.3 + 29.9) × 100) of the variation in the series is explained by the subsequent model. The strong seasonal component in those data comes out. The random walk component also implies that the current number of listeriosis cases depends on the past value plus an error term considered as white noise.

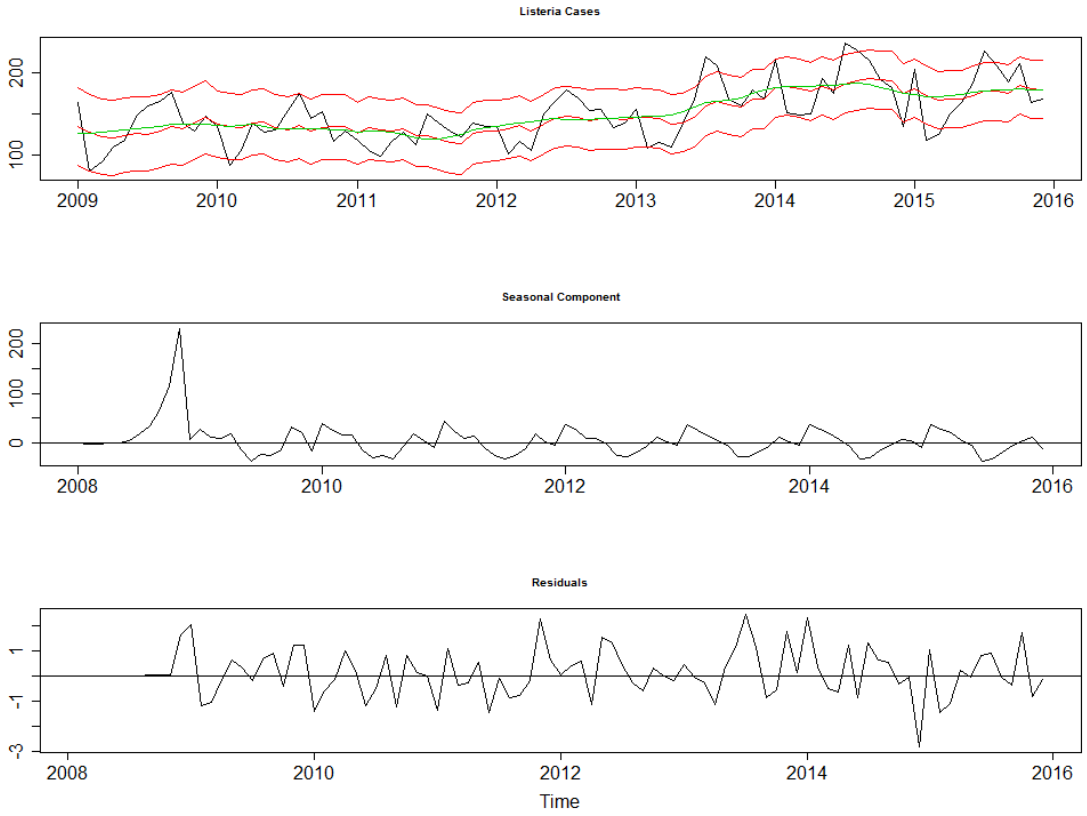
Figure 15 shows the original data expressed as human listeriosis cases and the original data expressed as listeriosis cases divided by the population, both in function of time. The two trends appear very similar, and it was decided to report the analysis using the listeriosis cases as the outcome only.



Top graph: human listeriosis cases; bottom graph: human listeriosis cases per 10,000,000 population.

Figure 15: Monthly human cases of confirmed human listeriosis in function of time observed in the EU/EEA, 2008–2015

Figure 16 shows the original data with several additional measures from the fitted model. The top panel shows the raw data with the filtered (red) and smoothed (green) fits. The filtered fits are the running version of the model for predicting time t given only time t-1 and past seasonal values. The smoothed values are the best prediction given all values from time $t = 1$ to time $t = T$ (thus why it is 'smoother'). The second panel in the plot shows the time-varying seasonal component. This shows that early in the series, it is hard to estimate the seasonality since it has a large variance compared with the later part of the series. In contrast, in the later part of the time series, the seasonality becomes more regular and easier to estimate. Finally, the last panel gives the standardised residuals.



Time

Top graph: cases with fitted random walk plus seasonal model and 95% credibility interval (red), and smoothed estimate (green). Middle graph: Seasonal component of the *Listeria* time series. Bottom graph: Standardised residuals of the *Listeria* time series after removal of the seasonal component and the trend.

Figure 16: Monthly cases of confirmed human listeriosis in function of time observed in the EU/EEA with several additional measures from the fitted model, 2008–2015

These residuals are white noise, meaning that the residuals are uncorrelated (i.e. stable) and that forecasting is allowed since we can model the dynamics, i.e. shocks/noise/residuals are uncorrelated. Their autocorrelation functions have values showing no residual serial correlation. In a TSA, current values of a dependent variable can be based on both the current values of an explanatory variable and on the lagged (past period) values of the dependent variable (e.g. one month earlier (t-1)). If such a relation exists between residuals and past residuals, this indicates that there is no white noise. Ljung–Box tests of the serial correlations tests the null hypothesis that there is no correlation between the residuals and the residuals in previous months. This test resulted in a p value of 0.88 at lag one month which led to the conclusion that the null of no serial correlation could not be rejected; in other words that there is no violation of the white noise in the model. The same is true at the seasonal lag of 12 months with a p value of 0.37.

When the local linear growth model is estimated, the variance estimates are $V = 23.6$, $W = 189.2$, and $U = 3.37 \times 10^{-8}$. This means that there is nearly no variance in the trend term β_t. This model was rejected because the second order term explained nearly zero of the variance in the *Listeria* time series.

The random walk plus seasonal trend is therefore the appropriate model. This model was used to forecast the number of human listeriosis cases, as shown in Figure 17, indicating the stability of the number of cases in the EU/EEA.

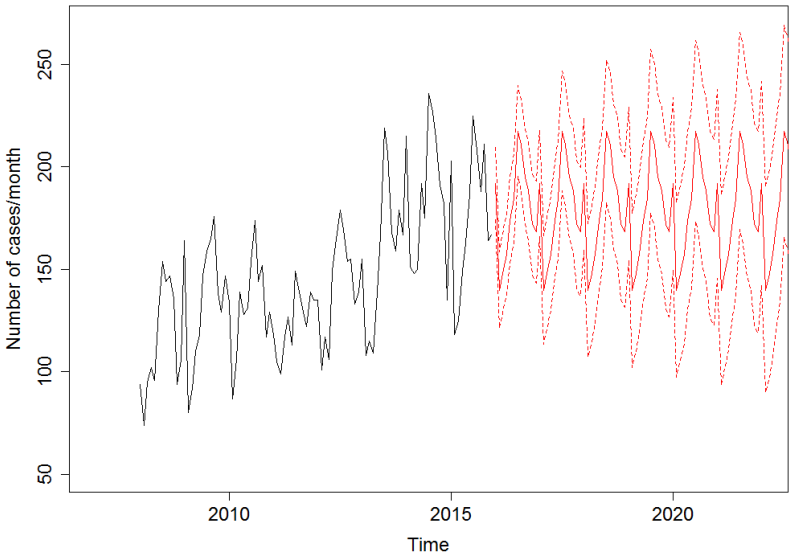


Figure 17: Cases of confirmed human listeriosis in function of time observed in the EU/EEA and future predictions based on the fitted random walk plus seasonal model, with the respective intervals based on one standard deviation, 2008–2015

These results are not consistent with the conclusions in the EU summary report by EFSA and ECDC (2016), which identified an increasing trend of confirmed human listeriosis cases during the period 2011–2015. This may be attributed to the difference in the time period considered, the countries included in the analysis, and the different analytical approaches being used. In the report, moving averages were used to assess the temporal trends at the EU level and linear regression was applied to test the significance of trends. A linear regression analysis does not address auto- and seasonal correlation, and assumes linearity and normally distributed residuals. The visual representation of the number of *L. monocytogenes* cases in the report with a moving average indicates a stable trend until 2013 and a change between 2013 and 2015, indicating non-linearity. The TSA accounted for auto- and seasonal correlation and other issues such as non-linearity. Obviously, this makes the detection of an increasing trend harder, i.e. requiring stronger evidence. It is noteworthy that the report also indicates (i.e. no *p* value reported as a statistical support) that the number of listeriosis cases stabilised in 2015.

3.5.2. Results of the disaggregated TSA

As mentioned earlier, the analysis of the aggregated 2008–2015 data did not result in the indication of an increasing trend of listeriosis incidence rates, probably partly a consequence of the presence of autocorrelation and seasonality. From this analysis it was unclear whether or not this absence of proof of a trend would also be valid when performing the analysis for subgroups.

Figure 18 shows the evolution of reported human listeriosis incidence rates in the EU/EEA between 2008 and 2015, by age and gender. The thick lines correspond to smoothed trend lines based on local regression. In Appendix B, the evolution has been shown for a selection of age groups using the same scale on the y-axis.

The thick lines correspond to smoothed trend lines based on local regression.

Figure 18: Evolution of reported human listeriosis incidence rates in the EU/EEA by age and gender, 2008–2015

As a first step, the incidence rate per person-month was calculated for the year 2008 as shown in Table 19. The human listeriosis incidence rate is statistically higher in the female 25–44 group than in the male 25–44 group, while the opposite is true for the 45–64, 65–74 and ≥ 75 age groups. The same differences are still noticed in 2015, but in that year the *Listeria* incidence rate is also statistically higher in the female than in the male 15–24 group.

Table 20 shows the results of a Poisson auto-regression. When no rho coefficients are reported, the fitted model is a Poisson regression with a population offset. The PAR(*p*) and Poisson regression estimates for the models with the offsets are the best fit for each gender–age series. The positive signs on the autoregressive coefficients indicate positive serial correlation when it is present. The trend (incidence rate) increases statistically when the *z* values are higher than 1.96 in absolute terms. Although one needs to be careful to use the monthly incidence rate ratios based on a Poisson regression in the presence of autocorrelation, the monthly relative average increase based on the latter regression model is shown in the last column in Table 20.

Statistically significant increasing trends in the incidence rates are noticed for the 25-44 and ≥ 75 age groups in the female population while a statistical increasing trend is only noticed in the ≥ 75 age group in the male population. For the female 45-64 and 65-74 age groups the increasing trend was borderline significant (z-values of 1.78 and 1.93, respectively, where z > 1.96 indicates significance at the 0.05 alpha level, Table 20). The 65–74 and ≥ 75 male groups needed PAR(2) models indicating that the presence of autocorrelation (two lags) resulted in overdispersion, with a possible influence on the significance of the trend as a result. The time plot for these age groups also shows a tendency to stay high or stay low, with an increase that only starts in 2012, which is also indicative of positive autocorrelation. This may be in relation to food shelf lives, i.e. food with long shelf life remaining on shelves, and therefore when cases arise due to consumption of such a food these cases are followed by cases later on.

Table 19: Incidence rates of human listeriosis (cases per million population and month) observed in the EU/EEA and the incidence rates by age–gender combination in 2008 and 2015

Year	Age group	Incidence in males (A)	Incidence in females (B)	Male/female incidence ratio (A/B)[b]	CI		*p* value[a]
2008	1–4	0.09	0.09	1.04	0.44	2.53	0.924
2008	5–14	0.04	0.02	1.88	0.77	5.03	0.166
2008	15–24	0.04	0.05	0.71	0.35	1.42	0.334
2008	25–44	0.05	0.12	0.43	0.30	0.61	< 0.001
2008	45–64	0.30	0.17	1.79	1.44	2.24	< 0.001
2008	65–74	0.89	0.50	1.78	1.44	2.20	< 0.001
2008	≥ 75	1.41	0.76	1.87	1.56	2.25	< 0.001
2015	1–4	0.03	0.02	1.83	0.34	14.81	0.491
2015	5–14	0.01	0.03	0.48	0.12	1.57	0.231
2015	15–24	0.03	0.11	0.30	0.15	0.58	< 0.001
2015	25–44	0.05	0.21	0.21	0.15	0.30	< 0.001
2015	45–64	0.32	0.23	1.38	1.14	1.67	0.001
2015	65–74	1.25	0.61	2.06	1.72	2.46	< 0.001
2015	≥ 75	2.20	1.30	1.70	1.49	1.94	< 0.001

CI: confidence interval.
(a): based on tests for independence for comparison of rates and test of independence two-sided *p* values calculated using mid-*p*.
(b): different rounded values are obtained by using the values shown in (A) and (B) due to the rounding to two decimals.

Table 20: Poisson autoregression model output with population offsets for the *Listeria* rates by age–gender combination[a]

Gender	Age group	Trend coefficient[b] (×100)	*z* value	Rho(1)[c]	Rho(2)[c]	Monthly % increase in incidence rate [d] (+ CI)
Female	1–4	<0.001	<0.01	—	—	Non-significant
	5–14	0.819	1.61	—	—	Non-significant
	15–24	0.532	0.97	**0.614**	—	Non-significant
	25–44	**0.610**	**2.90**	**0.347**	—	**0.64 [0.42,0.86]**
	45–64	*0.355*	*1.78*	***0.345***	—	*0.43 [0.23,0.64]*
	65–74	*0.313*	*1.93*	***0.245***	—	*0.30 [0.10,0.49]*
	≥ 75	**0.596**	**4.95**	**0.257**	—	**0.70 [0.55,0.84]**
Male	1–4	-0.111	-0.24	—	—	Non-significant
	5–14	-0.206	-0.41	—	—	Non-significant
	15–24	0.294	0.69	—	—	Non-significant
	25–44	0.021	0.11	—	—	Non-significant
	45–64	0.121	0.94	**0.269**	—	Non-significant
	65–74	0.238	1.41	0.195	0.156	Non-significant
	≥ 75	**0.353**	**2.76**	**0.123**		**0.50 [0.37,0.64]**

CI: confidence interval.
(a): Significant coefficients (alpha = 0.05) are shown in bold. Those with borderline significance are shown in italics.
(b): Coefficient obtained in the Poisson autoregressive [Par(p)] model, when Rhos are shown, otherwise coefficient for log(time) based on a Poisson model.
(c): The autocorrelation coefficient at lag *p*.
(d): Based on incidence rate ratio (monthly change) based on Poisson model.

The values in the last column in Table 20 are based on the monthly incidence rate ratios and estimated using a Poisson regression and are included for illustrative purposes. These indicate the increase expressed as a percentage, and can be interpreted as a proportional monthly increase, e.g. every month the incidence rate is augmented by 0.70% compared with the previous month for the female ≥ 75 group, while this is 0.50% for the males. Notice that the confidence intervals for the females ≥ 75 and males ≥ 75 overlap, indicating that the increase based on the Poisson model is not significantly different in the male group as compared with the female group.

A general conclusion is that some positive trends appear for the subgroups while this is not the case with the aggregated data. This is known as an ecological bias. Furthermore, the manifestation of, e.g.,

seasonality when aggregating temporal data, as seen in this study, has also been reported in other studies (Shellman, 2004).

3.5.3. Uncertainty analysis of the TSA

The sources of uncertainty in relation to assumptions and data for the TSA and the potential impact are shown in Table 21. The uncertainty related to the model fitting is quantitatively expressed using the CIs of the incidence rate changes. Apart from model fitting there are several additional uncertainty sources, which can lead to under- or overestimation of the observed trends.

The aggregated *L. monocytogenes* time series from January 2008 to December 2015 is short. Indeed, as a rule of thumb a minimum of 60 observations for a TSA is advised, and in the current study 80 observations are available. More information may affect the trend. With respect to the aggregated data, the most appropriate (dynamic linear) model was used because other potential models do not include autocorrelation or seasonal correlation and do not fully capture the dynamics of changing trends and a change point analysis did not indicate the presence of a change point. The inclusion of all the latter model characteristics reduces the possibility to detect a possible trend. No covariates were included in the aggregated model and homogeneity in group was assumed, which may hide the presence of trends in subgroups. This was less the case in the disaggregated data analysis, in which some of the uncertainty issues that were observed for the aggregated data were also noticed. Due to the available data the analysis and understanding of trends were performed using age and gender as proxies for susceptible populations and not including countries as a covariate. This is a limitation and means that the observed trends may hide trends among subgroups or be true for only a subset of the age–gender–country population.

Table 21: Potential sources of uncertainty identified in the time series analysis and qualitative assessment of the impact that these uncertainties could have on the incidence rate outcome and on the incidence rate trend in the EU/EEA between 2008 and 2015

	Input/parameter /model structure	Source of uncertainty	How uncertainty has been addressed	Direction of the effect on the incidence -/+[(a)]	Direction of the effect on the incidence trend -/+[(a)]
Data	Human listeriosis data	Under-ascertainment/under-reporting	A survey was performed in the EU/EEA countries about changes in diagnostic practices and in their national surveillance systems	+	+
		Classification of cases. Only laboratory-confirmed cases were included in the analyses	Not addressed	-	-/+
		Incomplete data. One Member State reported only aggregated data and was not in the original dataset (N = 46); three EU/EEA countries were excluded (N = 72); for some cases (N = 169) age, gender and/or month was unknown	Not addressed	-/+	-/+
Hypothesis /model	Model aggregated analysis	Model selection. The most appropriate model was used because other models did not include autocorrelation or seasonal correlation and did not fully capture the dynamics of changing trends	Other models, such as a change point were tried out, but did not result in a more appropriate model. Confidence intervals around the coefficients and incidence rate changes are indicative of uncertainty. The uncertainty may be overestimated and therefore more likely to not identify a trend than to identify a trend that is not there, given that the model used is relatively conservative	Not applicable	-
		No covariates are included in the aggregated model and homogeneity in group is assumed (same trend assumed in all Member States and comorbidity groups; uncertainty reduced by stratification by gender and age)	Country-specific analyses were not conducted in this Opinion. Analyses by gender and age were conducted elsewhere in this opinion	Not applicable	-/+
		Short time series for TSA (2008–2015)	Not addressed	Not applicable	-/+
		Influential data points (outbreaks included)	Not addressed	Not applicable	-/+

	Input/parameter /model structure	Source of uncertainty	How uncertainty has been addressed	Direction of the effect on the incidence -/+[(a)]	Direction of the effect on the incidence trend -/+[(a)]
Hypothesis /model	Model disaggregated analysis	See model aggregated analysis except for the covariates age and gender that are included	See model aggregated analysis except for the covariates age and gender that are included. The models are relatively conservative albeit less conservative than the model for the aggregated data since seasonality was not needed for analysing the disaggregated data	Not applicable	-
		PAR(p) model may only approximate low order serial correlation in the data	Seasonal correlation was investigated but not present at the disaggregated data level	Not applicable	+

N: number of cases; PAR(p): Poisson autoregressive model.
(a): + means that the (real) outcome/effect is possibly overestimated, - means that the (real) outcome/effect is possibly underestimated.

3.5.4. Conclusions of the TSA in the EU/EEA, 2008–2015

- The TSA of the aggregated 2008–2015 confirmed listeriosis data did not result in the indication of an increasing trend of listeriosis incidence rates in the EU/EEA. This is partly a consequence of the presence of changing dynamics, autocorrelation and strong seasonality in the aggregated analysis, and an analysis capturing all these components. This is in contrast with analyses of data for certain age–gender groups which revealed clear trends and where some of the aforementioned characteristics were present to a lesser extent.
- For females, the incidence rate of confirmed human listeriosis significantly increased for the 25-44 and ≥ 75 age groups in this time period with a monthly increase estimated at 0.64% and 0.70%, respectively. For the female 45-64 and 65-74 age groups the increasing trend was borderline significant with a monthly increase estimated at 0.43% and 0.30%, respectively.
- For males, the incidence rate of confirmed human listeriosis cases increased significantly for the ≥ 75 age group only with a monthly increase of the incidence rate estimated at 0.50%.
- Some differences between females and males in the increases of the incidence rates were noticed, e.g. for certain age groups, (borderline) increases were noted in some female groups and not in the male groups.
- Based on a comparison of the incidence rate in 2008 a significantly higher listeriosis incidence rate was noticed in males than females in the 45–64, 65–74 and ≥ 75 age groups, whereas the incidence rates were higher for females than males in the 25–44 age group. These differences remain similar by 2015, except that the difference in the 25–44 age group had increased significantly and that the incidence rates were higher for females than males in the 15-24 age group.
- The highest incidence rate was seen in the ≥ 75 group resulting in 2015 in an incidence rate of 2.20 and 1.30 cases per month per million persons for the males and females, respectively. All other age and gender groups have lower incidence rates.
- The uncertainty related to the model fitting is quantitatively expressed using the CIs of the incidence changes. Apart from model fitting there are several additional uncertainty sources, which can lead to under- or overestimation of the observed trends. Due to the available data, the analysis and understanding of trends were performed using age and gender as proxies for susceptible populations and not including countries as a covariate. This is a limitation and means that the observed trends may hide trends among subgroups or be true for only a subset of the age–gender–country population.

3.6. Evaluation of factors that may explain the epidemiological trend of human listeriosis

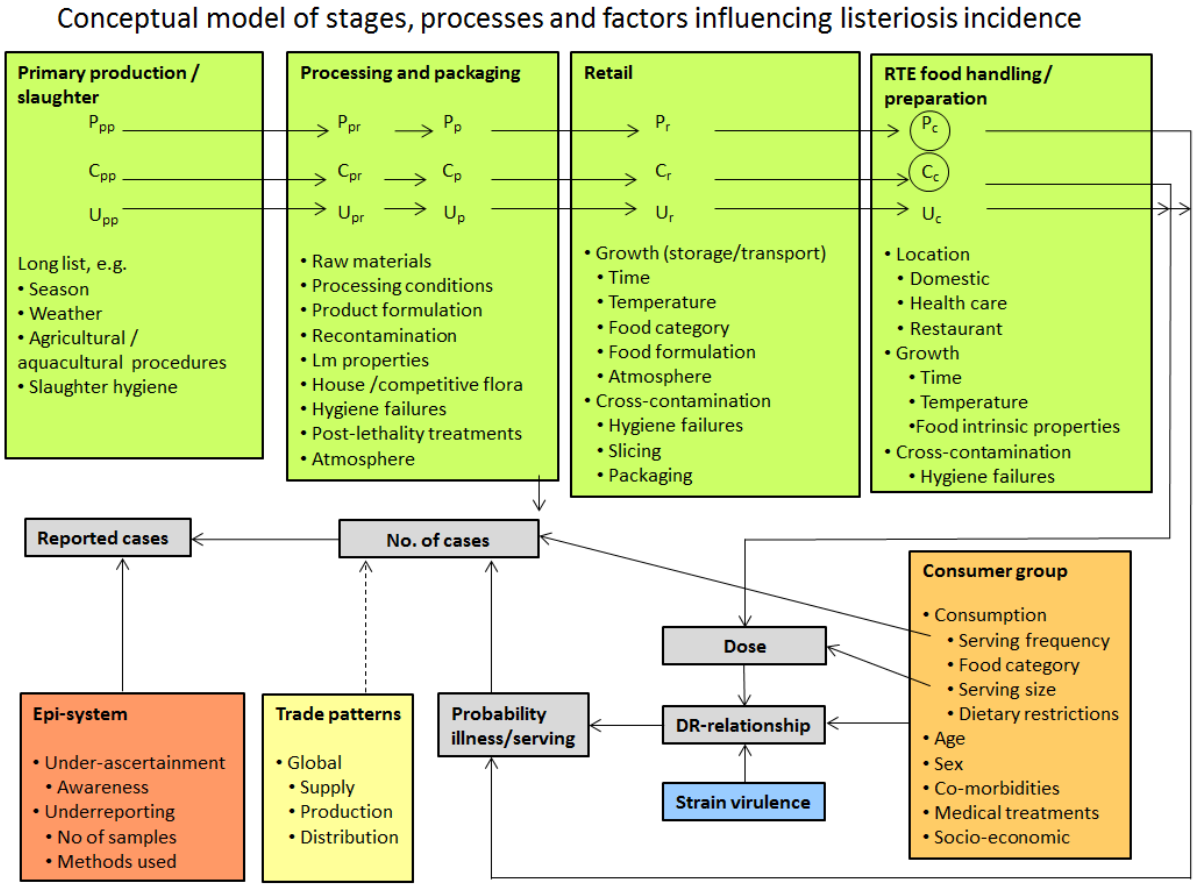
As described in the methodology section (Section 2.2.3), potential factors that may explain the epidemiological trend were identified by the working group via a conceptual model and were evaluated as AQs in three steps. First, an importance analysis was used to evaluate the most important factors and their potential impact on the number of predicted cases using the gQMRA model (see Section 2.2.4). The second step was to evaluate the empirical evidence, i.e. the indicator data, to investigate the support for a change in the factor during the time period. In the third step, an evidence synthesis of the TSA, the importance analysis, indicator data and the uncertainty analyses was made. Based on the outcome of this evaluation conclusions were drawn, with uncertainties described, on the impact of the different factors on the human listeriosis incidence rates.

The starting point for identifying the relevant factors was a simplified conceptual model for *L. monocytogenes* contamination in the food chain and for the reported incidence rates of human illness (Figure 19). It should be pointed out that the factors identified can act alone or in combination, and that the influence of some factors cannot be evaluated explicitly but are considered indirectly through their effects on prevalence and concentration at retail or the other factors in the model.

Contamination levels and prevalence of *L. monocytogenes* in RTE foods at different stages in the food chain and related influencing factors and processes are shown in green boxes. For instance, growth

rates depend both on intrinsic properties of the food such as the formulation of the RTE food, and on extrinsic factors such as storage temperature. Prevalence and concentration might change when the size of the food unit (U) is changing, along the different steps of the food chain (e.g. Nauta (2002)). The properties and type of the RTE food and the consumption habits of the individuals in the consumer group of interest (orange box) influence the ingested dose and the relevant DR relationship (grey boxes). The DR model is dependent on the population group considered and it is assumed that, for example, medical treatments and the virulence of *L. monocytogenes* strains (blue box) may affect the vulnerability of the consumer and lead to an adjustment of the DR model. Further, the number of cases is a function of the probability of illness per serving and the number of servings consumed by the individuals in the consumer group, i.e. a function of the serving frequency. The number and distribution of cases may also be different if the pattern of trade of ingredients and/or the final product are global, regional or local (yellow box). Finally, the influence of the national surveillance system (Figure 19, red box) is reflected in the reported number of cases which may be less than those actually occurring, dependent on under-ascertainment and under-reporting. Under-ascertainment refers to symptomatic cases not contacting health services, whereas under-reporting refers to known infected individuals whose disease status is misdiagnosed or fails to be reported to the organisation responsible for surveillance (van Lier et al., 2016).

To be able to unambiguously evaluate the different factors a general assessment question was formulated as: 'What contribution (= impact) did any change in factor *x* make to the change of cases/incidence rates of human listeriosis in the EU and EEA in the time period 2008–2015?'



C: concentration; Lm: *Listeria monocytogenes*; P: prevalence; U = food unit size, which may affect the distribution of Lm, i.e. P, and C, considerably. The subscript for C, P and U refers to the production stage.

Figure 19: A conceptual model describing important factors and processes related to different stages in the food chain (green boxes), consumers (orange box), the epidemiological system (red box), and trade patterns (yellow box) and how they combine (grey boxes and arrows) to influence *Listeria monocytogenes* contamination, ingested dose, dose–response relationships and the incidence rates of reported human listeriosis

3.6.1. *Listeria monocytogenes* generic QMRA (gQMRA) model: baseline

In order to test the possible implication of certain factors to the increase of human listeriosis cases and incidence rates after 2011, a baseline gQMRA model was run with the prevalence and initial concentration of *L. monocytogenes* in RTE foods and the consumption pattern assumed to represent the situation during the period 2010–2011. The baseline gQMRA model considers option 3 for the initial concentration of *L. monocytogenes* in RTE foods. The outputs are presented in Table 22. The *L. monocytogenes* prevalence in the first column was calculated by weighting the prevalence observed in the different RTE food categories by their consumption in each population group; therefore providing an average prevalence across all food categories for each population group. Consequently, the differences in prevalence between the groups are due to the different consumption patterns; the estimated prevalence is higher in the age group ≥ 75 years old. The total number of cases per year estimated by the model is 1,523, and this is as expected, close to the average reported number of cases in 2008–2011, i.e. 1,521 cases.

Table 22: Output of the baseline gQMRA model for the subpopulations included in the time series analyses

Population group (gender and age in years)	Prevalence[(a)]	Total number of eating occasions per year (A)	Risk per eating occasion (B)	Cases per year (A×B)
Female 1–4	0.03516	2.90E+09	1.73E-09	5
Male 1–4	0.03647	3.07E+09	2.37E-09	7
Female 5–14	0.02567	6.64E+09	8.29E-10	5
Male 5–14	0.02578	7.58E+09	7.62E-10	6
Female 15–24	0.02806	6.27E+09	3.86E-09	24
Male 15–24	0.02466	8.74E+09	9.39E-10	8
Female 25–44	0.02503	1.81E+10	6.49E-09	117
Male 25–44	0.02526	2.50E+10	1.72E-09	43
Female 45–64	0.02768	2.02E+10	6.60E-09	134
Male 45–64	0.02739	2.68E+10	8.65E-09	232
Female 65–74	0.03371	8.98E+09	1.65E-08	148
Male 65–74	0.0332	9.75E+09	2.40E-08	234
Female ≥ 75	0.04045	1.01E+10	2.58E-08	260
Male ≥ 75	0.04286	9.06E+09	3.31E-08	300

For the initial concentration of *L. monocytogenes* in the RTE foods, fish distributions from BLS data, and meat and cheese distributions from US data were used (option 3). One million iterations were used.

(a): The *L. monocytogenes* prevalence was calculated by weighting the prevalence observed in the 13 RTE food subcategories/packaging conditions by their consumption in each population group.

Table 23 summarises the distributions of concentration at retail level and at time of consumption. The frequency of a contaminated food having a concentration higher than 5 $\log_{10}$ CFU/g, after one million iterations, increases during storage by overall less than 1% of a unit.

Table 23: Probabilities of exceeding certain *L. monocytogenes* concentrations in ready-to-eat (RTE) food categories at retail level (initial) and at time of consumption estimated using the gQMRA model

RTE foods	Limits in $\log_{10}$ CFU/g	ROP packaging		Normal packaging		ROP	Normal
		Initial	At time of consumption	Initial	At time of consumption	Ratio (at time of consumption/initial)	
Cold-smoked fish	2	0.0718515	0.0801105	0.0719185	0.0744645	1.11	1.04
	3	0.02316	0.027376	0.023388	0.024621	1.18	1.05
	4	0.003465	0.005071	0.0035125	0.003904	1.46	1.11
	5	0	0.000282	0	0.0000215	NA	NA
Hot-smoked fish	2	0.109821	0.115353	0.110008	0.112133	1.05	1.02
	3	0.048605	0.0518215	0.0486695	0.049925	1.07	1.03
	4	0.01593	0.017546	0.0159055	0.0165435	1.10	1.04
	5	0.0024265	0.002996	0.002454	0.0026415	1.23	1.08
Gravad fish	2	0.040043	0.046614	0.039865	0.0660235	1.16	1.66

	3	0.008711	0.011015	0.0086345	0.0210965	1.26	2.44
	4	0.000963	0.0015295	0.0009855	0.006628	1.59	6.73
	5	0.0000195	0.000143	0.000027	0.002725	7.33	100.93
Cooked meat	2	0.0613245	0.0678085	0.061527	0.0690115	1.11	1.12
	3	0.02456	0.0281025	0.0247935	0.028823	1.14	1.16
	4	0.00708	0.008723	0.007179	0.0091095	1.23	1.27
	5	0.0008975	0.0014355	0.000914	0.0015915	1.60	1.74
Sausage	2	0.061126	0.067398	0.0614355	0.06721	1.10	1.09
	3	0.0242485	0.027637	0.0247555	0.027795	1.14	1.12
	4	0.0070025	0.008518	0.0070405	0.0084225	1.22	1.20
	5	0.0008785	0.0013825	0.0008925	0.0013355	1.57	1.50
Pâté	2	0.0614095	0.0654205	0.061277	0.0691885	1.07	1.13
	3	0.0245955	0.026761	0.0246855	0.028979	1.09	1.17
	4	0.007037	0.007976	0.0071045	0.0090605	1.13	1.28
	5	0.0008555	0.00114	0.0009105	0.001562	1.33	1.72
Soft and semi-soft cheese	2	0.0227085	0.024073			1.06	
	3	0.009779	0.0104335			1.07	
	4	0.003453	0.003769			1.09	
	5	0.0008685	0.0010045			1.16	

NA: not applicable; ROP: reduced oxygen packaging.
Monte Carlo simulation with 2 million iterations was used. For the initial concentration of *L. monocytogenes* in the RTE foods, fish distribution from the baseline survey data, and meat and cheese distributions from US data were used (option 3).
Estimation of the probability of exceeding certain limits of concentration and ratio of the probabilities at time of consumption and initial concentration. The darker the shaded colour, the higher the probability.

Combining the consumption patterns for different food categories the distribution of exposure doses is derived for each subpopulation. When eating a contaminated food, the probability of being exposed to a dose higher than 5 $\log_{10}$ CFU varies between 1.34% and 2.06%. Considering the overall prevalence of contaminated RTE food categories, the probability of exceeding an exposure dose of 5 $\log_{10}$ CFU is between 0.042% and 0.078%.

The overall impact of growth on the risk of human listeriosis was assessed by comparing the baseline expected number of cases with a scenario where growth was excluded. The expected number of cases in the absence of growth is presented in Figure 20. Without growth, the total number of cases (sum of the cases per subpopulation) was reduced from 1,523 (Table 22) to 953 (Figure 20) showing that absence of growth from retail onwards may save, on average, 570 cases (37%, 570/1,523).

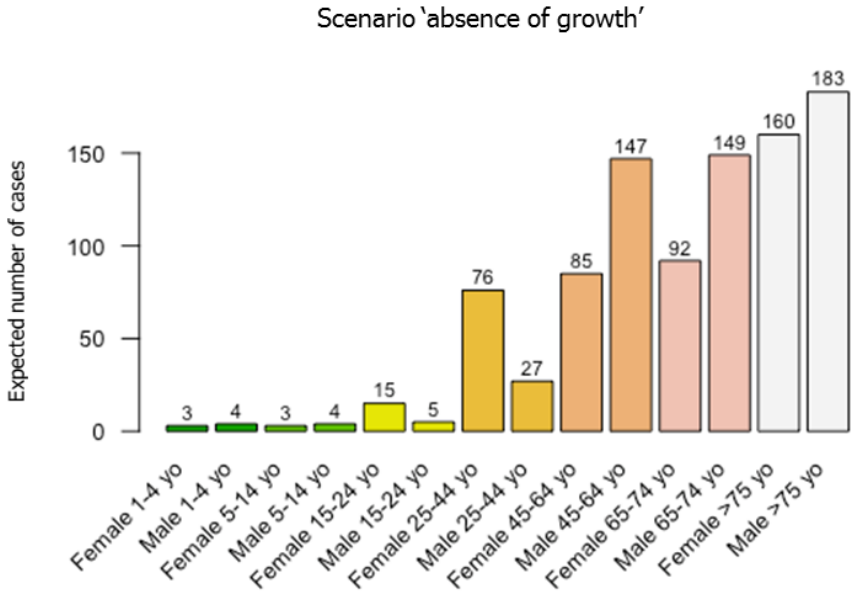
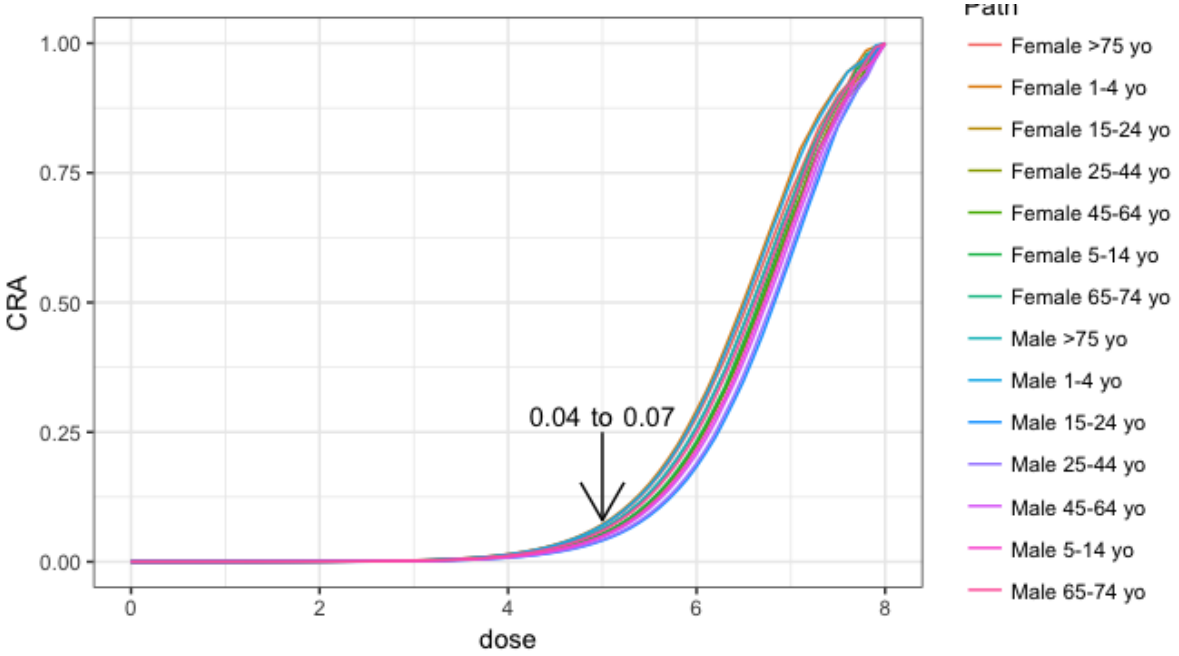


Figure 20: Expected number of human listeriosis cases per subpopulation and per year in the EU/EEA (1 million iterations) with a scenario 'absence of growth'

The DR model was applied to calculate the risk per serving for each subpopulation and the cumulative risk attribution was calculated for each possible dose of exposure. The cumulative attribution risk for a specific dose (x) is the proportion of human listeriosis cases attributable to doses lower than or equal to x. The doses lower than or equal to 5 $\log_{10}$ CFU could be responsible for 4.08% to 7.22% of the total cases, meaning that 92.78% to 95.02% of cases are attributable to exposures with a dose higher than 5 $\log_{10}$ CFU (Figure 21). Considering this result, the total number of human listeriosis cases would be very sensitive to the fraction of exposure with high doses (> 5 $\log_{10}$ CFU) which corresponds to an average concentration of 3.3 $\log_{10}$ CFU/g (when considering an average serving size of 50 g).



The cumulative attribution risk for a specific dose (x) is the proportion of human listeriosis cases attributable to doses lower or equal to x.

Figure 21: Cumulative risk attribution of human listeriosis per subpopulation for the considered ready-to-eat food subcategories

3.6.2. gQMRA model: importance analysis

The factors evaluated had an impact on the outcome in a similar way for the different subpopulations. The risk change is presented in Figure 22 as a multiplication factor relative to the baseline model.

For a doubling in risk due to the most common time of consumption, the mode of the proportion of the remaining shelf life is investigated. It is concluded that the timing of consumption needs to be shifted by 0.5 to around 0.8 instead of the baseline of 0.3 (Figure 22a). To increase the risk by a factor of 2 (Figure 22b), the maximum increase of the listeriosis incidence rates observed in the TSA, consumers need to consider the maximum acceptable remaining shelf lives of RTE products to be 2.4 times the recommended instead of the 1.1 times as assumed in the baseline scenario. This is under the assumption that the most common time of consumption still occurs at a time point corresponding to 0.3 of remaining time of the recommended shelf life. Similarly, a maximum remaining shelf life of 1.4 would increase the incidence rate by a factor of 1.13 (Figure 22b).

The effect of the mean storage temperature (which influences growth) on the risk of listeriosis is perhaps less than expected (Figure 22c). A doubling in incidence rate results when the mean storage temperature increases from 5.9°C in the baseline scenario to between 9 and 10°C.

The gQMRA model output was very sensitive to a shift in the *L. monocytogenes* maximum population density. A shift of less than 0.5 $\log_{10}$ CFU/g would result in a doubling of the risk (Figure 22d). Even a small shift of 0.2 $\log_{10}$ CFU/g resulted in an increase of risk by a factor of 1.4 (Figure 22d). An increase in this parameter could also be interpreted as a shift of non-compliant samples to higher concentrations.

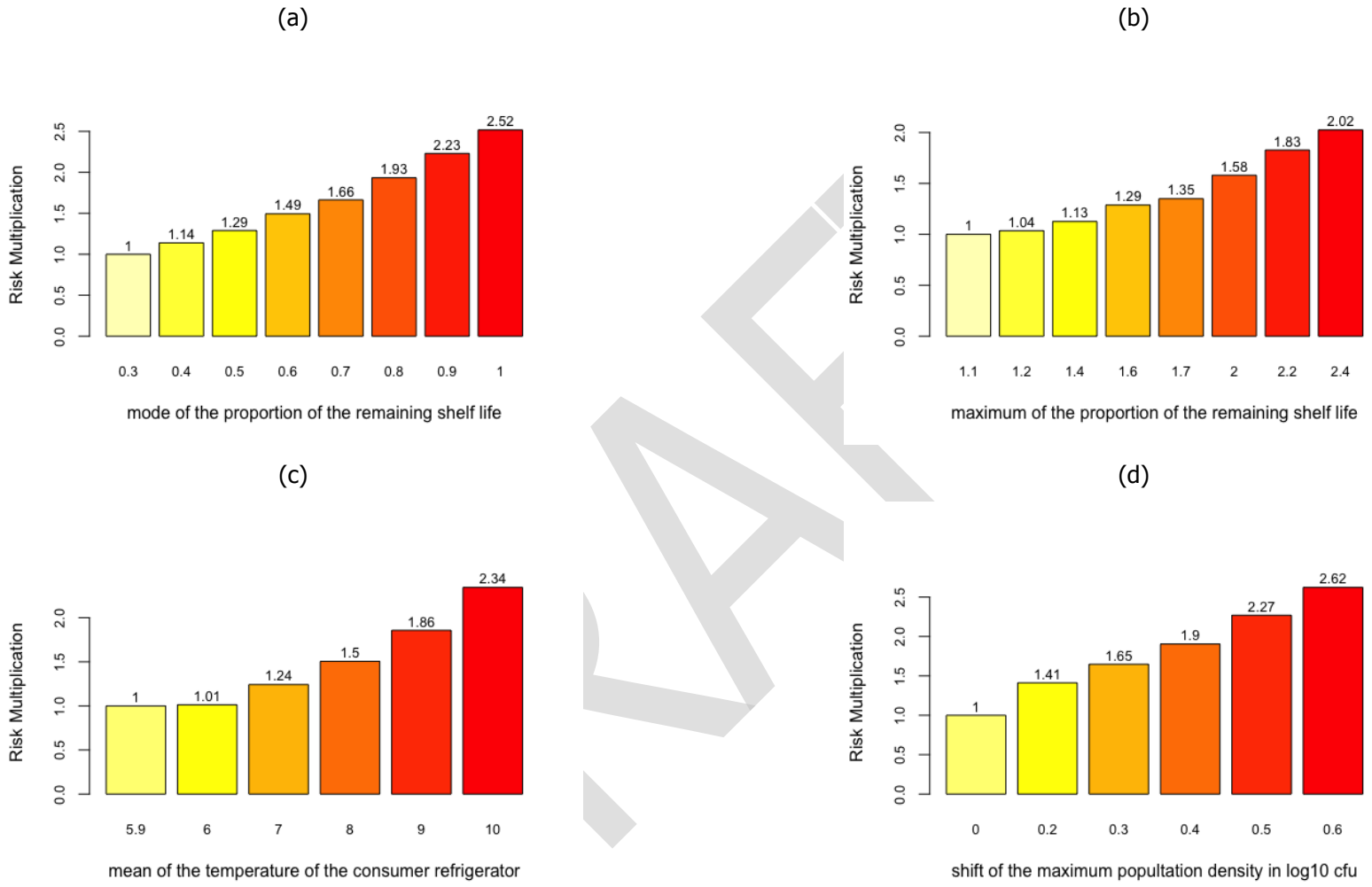


Figure 22: Increase in risk of human listeriosis as a function of (a) the mode of the proportion of the remaining shelf life used to store ready-to-eat (RTE) food in the consumer refrigerator (using simulations with a maximum proportion equal to 1.1); (b) the maximum of the proportion of remaining shelf life time used to store RTE food in the consumer refrigerator (using simulations with a mode of the proportion equal to 0.3); (c) the mean of the mean temperature of the consumer refrigerator; (d) the maximum population density shift

3.6.3. Indicator data

Prevalence

EFSA monitoring data

The monitoring data have several limitations for the purpose of evaluating any changes in prevalence of *L. monocytogenes* in RTE foods. As described in Sections 2.1.2. and 3.3.2., these data are evaluated in accordance with the *L. monocytogenes* microbiological criteria applying certain assumptions that have been spelled out in the latest EU summary report (EFSA and ECDC (2015)). Boelaert et al. (2016) stated that 'In essence, food chain control data are compliance checks and are collected with the aim to install an early warning and initiate control measures. Although they can be used for trend watching (which covers general observations of harmonized or non-harmonized data for possible trends), these data are unsuitable for trends analyses, because a reference (study) population is mostly absent and because the sampling is risk-based and thus, non-representative.'

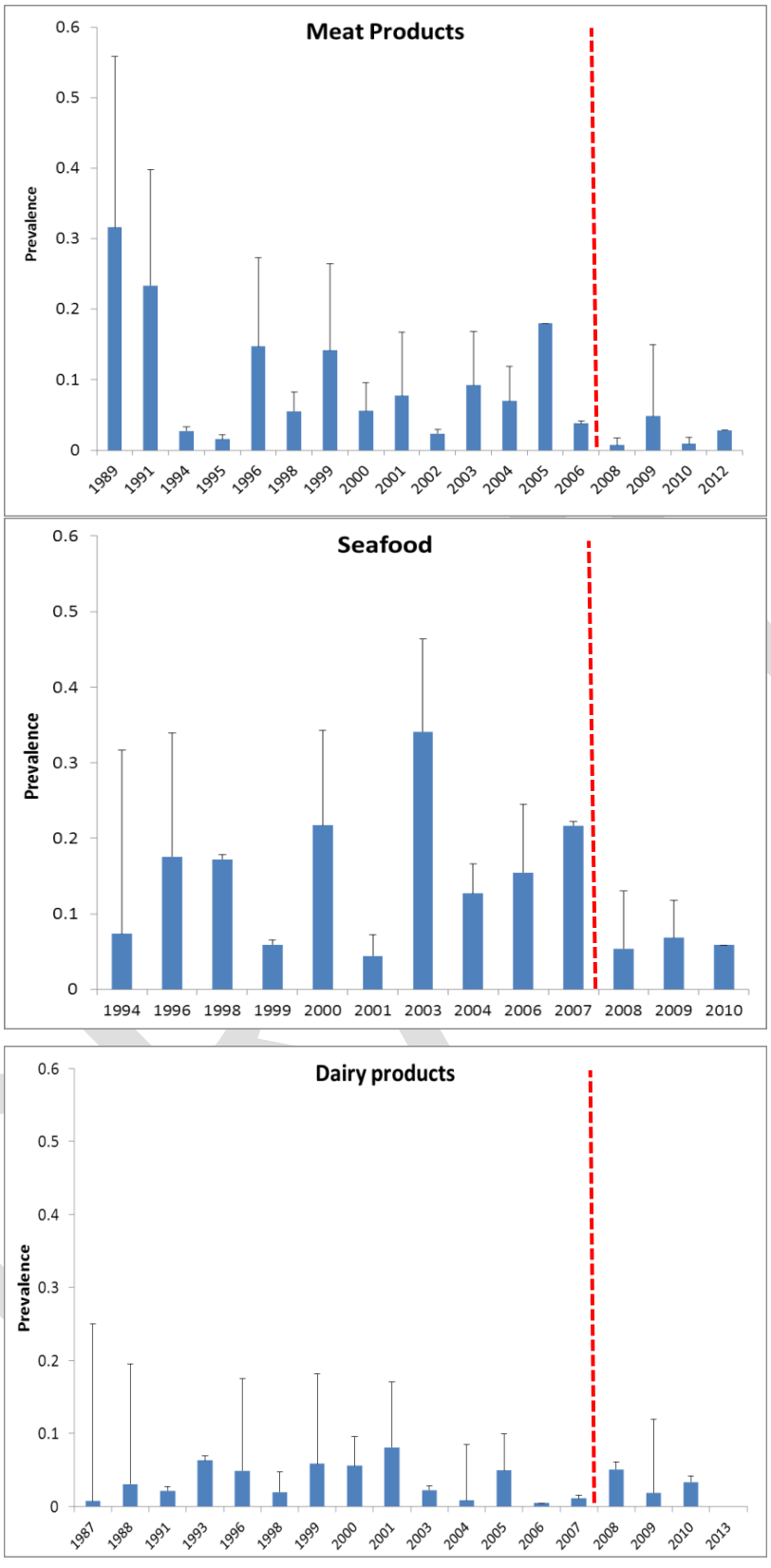
For some subcategories the results presented in Figure 11 represent a substantial number of samples, e.g. at processing in 2014, a total 40,853 samples of RTE products of meat origin other than fermented sausage were reported, and at retail in 2008 for the same category 16,653 samples were reported. Still, the results are sensitive to the type of samples, the sampling schemes and the number of Member States reporting in a single year. At the low prevalence reported (a few per cent or even below 1 per cent) a large number of samples is needed to make it possible to draw conclusions about any differences between years and subcategories. Thus, the data may at best indicate the magnitudes of the prevalence in the food subcategories during the time period but it should be pointed out that the results between years is sensitive to the amount of sampling, the Member State reporting and varying sampling strategies over years even within a Member State. Another issue is that the available data relate to compliance of products with the microbiological criterion, which is absence at 25 g at processing and 100 CFU/g at retail. As such, prevalence is potentially directly linked to non-compliant products at the processing stage, whereas at retail, prevalence is expected to be higher than the non-compliance. For these reasons, supporting evidence for a trend in prevalence cannot be concluded for samples taken at retail. At processing, the same limitations mentioned above apply but some observations can be made. The *L. monocytogenes* non-compliance in fishery products from 2013 to 2015 appears lower than that from 2008 to 2012. Similarly, non-compliance in meat products other than sausages from 2010 onwards appears lower than during the preceding years. For the other subcategories incomplete data and variable percentages of non-compliance over the years are observed (i.e. sausages and dairy products).

In conclusion, based on the monitoring data on percentages of non-compliant food items there is no evidence to suggest an increase of the prevalence or non-compliance of *L. monocytogenes* in RTE foods over time. The uncertainty of this conclusion is high due to the limitations associated with the data for the purpose of evaluating prevalence changes.

Literature data

Figure 23 shows the trend of prevalence with time for the period of conducting the studies presented in the extracted literature reports, for the three major RTE food categories.

The suitability of the data to evaluate trends is unclear and the observed prevalence varies over time in all food categories. The data do not support an increase in prevalence of *L. monocytogenes* in the three RTE food categories during the 2008–2015 time period but the uncertainty of the conclusion is high due to the limitations associated with the data.



Red vertical line shows the beginning of the targeted period of concern for the present Scientific Opinion extending from 2008 onwards. This period is also characterised by scarcity of data.

Figure 23: Prevalence data for *L. monocytogenes* in the three major RTE food categories based on literature data for the period 1989–2013

Concentration

The RASFF data were further analysed in relation to the year of reporting. Figure 24 presents a summary trend graph showing the change in the average value and the variability of the *L. monocytogenes* concentration for 'fish and fish products.' The highest average concentrations (3.01 $\log_{10}$ CFU/g) were reported in 2008. Notifications during 2014 showed the lowest average concentration (2.17 $\log_{10}$ CFU/g). The highest maximum concentrations were reported for defrosted smoked salmon (210,000 CFU/g, 2011), mackerel fillets with pepper (110,000 CFU/g, 2009), chilled salmon (85,000 CFU/g, 2014), skinned juniper-smoked trout fillets (44,000 CFU/g, 2008) and smoked salmon (40,000 CFU/g, 2010).

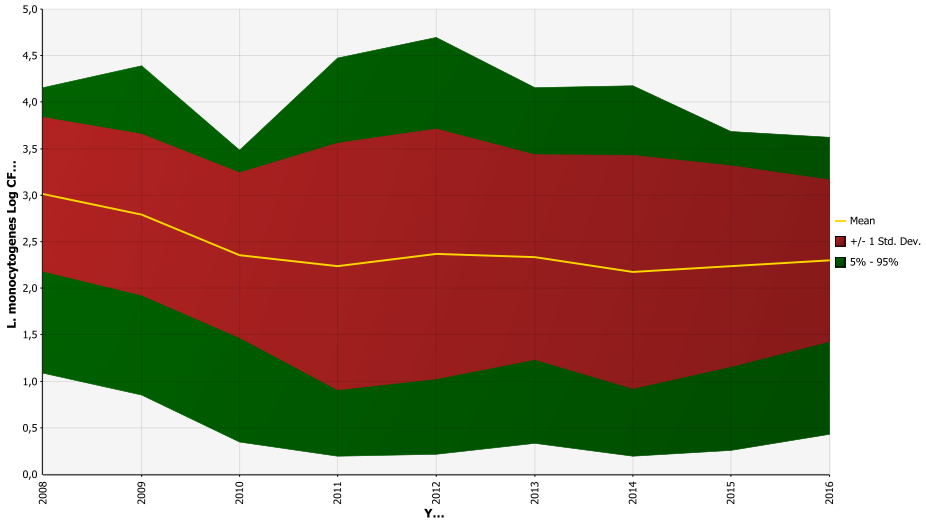


Figure 24: Summary trend graph for *Listeria monocytogenes* concentration in 'fish and fish products' reported in RASFF notifications for the years 2008–2016

The summary trend graph showing the change in the average value and the variability of the *L. monocytogenes* concentration in 'meat and meat products other than poultry' is presented in Figure 25. The highest average concentrations (2.74 $\log_{10}$ CFU/g) were reported in 2011. Notifications during the year 2013 showed the lowest average concentration (1.21 $\log_{10}$ CFU/g). The highest maximum concentrations were reported for smoked bacon (56,400 CFU/g, 2012), salami (31,000 CFU/g, 2011), chilled beef stew (15,000 CFU/g, 2016), cream pâté (15,000 CFU/g, 2011) and black pudding sausage (14,557 CFU/g, 2010).

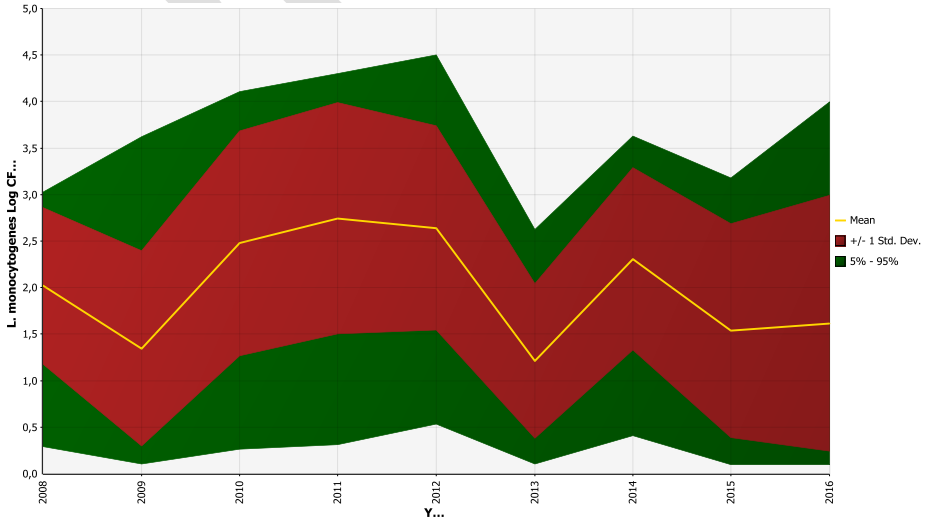


Figure 25: Summary trend graph for *Listeria monocytogenes* concentration in 'meat and meat products other than poultry' reported in RASFF notifications for the years 2008–2016

The summary trend graph of *L. monocytogenes* concentration for 'milk and milk products' is presented in Figure 26. The highest average concentrations (3.13 $\log_{10}$ CFU/g) were reported in 2008. Notifications during 2013 showed the lowest average concentration (1.23 $\log_{10}$ CFU/g). The highest maximum concentrations were reported for chilled gorgonzola (1,800,000 CFU/g, 2015), raw buffalo milk cheese (740,000 CFU/g, 2008), raw milk cheese (200,000 CFU/g, 2013), gorgonzola cheese (190,000 CFU/g, 2014) and cheese (140,000 CFU/g, 2014).

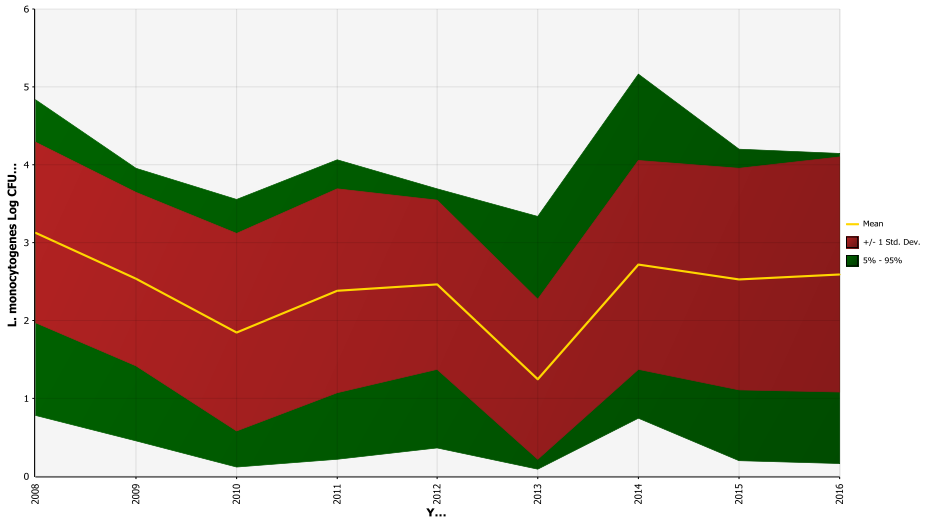


Figure 26: Summary trend graph for *Listeria monocytogenes* concentration in 'milk and milk products' reported in RASFF notifications for the years 2008–2016

In conclusion, among foods involved in RASFF notifications, high *L. monocytogenes* concentrations can be found in the RTE food categories such as 'fish and fish products,' 'meat and meat products other than poultry;' and 'milk and milk products.' There is no obvious trend over the period 2008–2016. It should be noted that the variability in concentration is high while the uncertainty related to the fact the RASSF data are not based on a systematic sampling procedure should be taken into account.

Consumption

EFSA consumption database

The change in the number of servings per year for the age group over 65 years old was estimated based on the available data in four northern European countries. To make the comparison of any changes over time easier to observe, the difference between means is shown in Appendix G.

Overall, there is some support for an increase in terms of the number of servings of RTE foods but the pattern is different for the different countries. For instance, Denmark has an increase in the number of servings for most food categories for both genders whereas there is weak support for this in the Netherlands. The time interval between the two surveys is longest for the Swedish data and for some food categories the results are based on few consumption events. Cooked meat and soft and semi-soft cheese (both genders) and smoked fish (males) indicate an increase in several countries.

In conclusion, there is some support for an increase in the number of servings for some food categories (cooked meat, soft and semi-soft cheese) as well as decreases in others, but it is not possible to draw any general conclusions due to the few countries involved and other limitations of the data.

Food and Agriculture Organization data

In order to get a rough estimate of the possible smoked salmon consumption in the EU for a recent period (2003–2013), FAO data were accessed, through the application FishstatJ and the workspace FAO Fishery and Aquaculture Statistics.[36] Three trade flows, i.e. production, import and export data (weights in tonnes) were obtained for EU countries for the years 2003–2013 (production data were not available for all countries). Subsequently, a calculation was made in which production and import weights were added for each year and country and export weights were subtracted from this sum. When any of the three trade flows variables were generally available for a country, but missing for some years, all data for that combination of country and year were removed from the calculation. Also, sometimes the outcome of the above calculation was a negative number. In those cases, this outcome was substituted by zero. This was the case for some years for several countries and for all the years for Lithuania and Poland. Some additional assumptions were made for the calculations of the overall numbers, for example values reported as over zero but less than half a tonne were substituted by zero, while data that were reported as having been estimated by FAO were used in the same way as the rest of the reported data. For more insights on the data used and more specific assumptions used in the FAO datasets, the reader is referred to the original FAO source.

This result, which can be viewed as a proxy for consumption (in tonnes) was added for all EU countries (26 countries included, while Lithuania and Poland were excluded, as explained above) for each year of the above-mentioned time period, and it showed an increasing tendency from year to year. Indeed the overall sums (in tonnes) were: 2003: 80,354; 2004: 78,648; 2005: 86,781; 2006: 91,884; 2007: 102,238; 2008: 105,396; 2009: 117,891; 2010: 120,408; 2011: 133,995; 2012: 145,636; 2013: 149,232.

In conclusion, there is an indication that the proxy for consumption of smoked salmon has increased by more than 40% during the 2008–2013 period. The uncertainty is high (proxy, only smoked salmon, missing after 2013).

Surveillance

The EU-level surveillance of invasive human listeriosis was established in 2008 and since then countries have aimed to improve their national surveillance systems. A short consultation of the FWD-Net contact points among those countries that are included in the EU-wide TSAs revealed that nine countries had improved their national reporting systems either slightly (N = 5) or moderately (N = 4), whereas eight countries replied to the question with 'not at all.'

Two countries with a relatively high level of case reporting have improved their national surveillance systems to the extent that it may have influenced the overall increase. Germany reported a change in the case definition to a more sensitive one and an increase in the reimbursements for diagnostic tests by the insurance companies. Spain has improved the partial surveillance coverage (i.e. more regions reporting human listeriosis) from 25% in 2009–2012 to 30% in 2013 and 45% in 2014–2015 (EFSA and ECDC, 2016). In Germany, the cases increased from 2008 to 2015 by 90% and in Spain the increase was 134% (Appendix D).

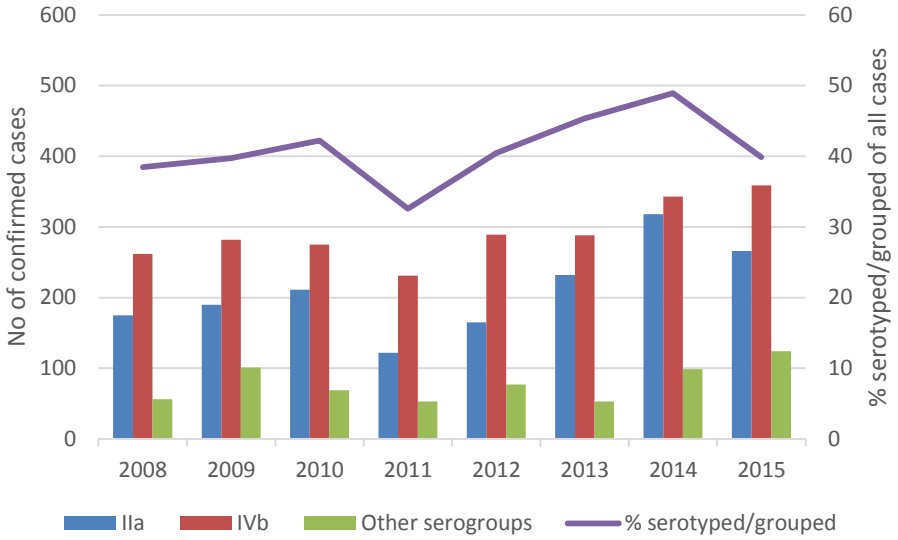
Thirteen countries (42%) responded to the questionnaire targeted to microbiologists. In 2008–2015, the indication of testing for *L. monocytogenes* has not changed for pregnant women (N = 8) and other patients (N = 10) (remaining five and three replies respectively were 'don't know'). Eight countries replied that the diagnostic methods have changed 'slightly' (N = 3), 'moderately' (N = 3), or 'very much' (N = 2). Five countries have introduced PCR-based detection of *L. monocytogenes* in liquor and blood while MALDI-TOF has been adopted by clinical microbiologists in three countries.

To conclude, there have been some changes in the surveillance systems, in particular for some countries with a relatively high level of reporting, which may have contributed to the increasing trend in confirmed listeriosis cases in the EU/EEA. There are some changes in the diagnostic methods but they are not expected to have contributed to the trend.

[36] FAO. ©2016. Fishery and Aquaculture Statistics. Global Fisheries commodities production and trade 1976-2013 (FishstatJ). In: FAO Fisheries and Aquaculture Department [online or CD-ROM]. Rome. Updated 2016. http://www.fao.org/fishery/statistics/software/fishstatj/en

Virulence/pathogenicity/serogroups

The new insights into the relation between clonal complexes and virulence, and new sequencing data may allow the hypothesis of a shift to more virulent and/or pathogenic *L. monocytogenes* strains to be thoroughly addressed but these data were not yet available. As a proxy, data on CFRs and serogroups over the time period were used. If cases under 1 year old are excluded, CFRs increase by age and this is detected in almost every year for age groups over 45 years for both sexes. In some years, however, the age group 45–64 years may show higher CFR values than those over 65 years, indicating the potential impact of underlying conditions (Tables 24 and 25). The human data from The European Surveillance System indicated that infection with serogroup IVb among middle-aged and elderly people increased the likelihood of a fatal outcome (Table 10). However, although variable, there is no apparent increase in the CFRs in these age–gender groups over the time period (Tables 24 and 25), even though the number of serogroup IVb cases reported also appeared to increase (Figure 27). The data in this figure are based on reporting in the four Member States with stable reporting of serotypes/serogroups over time, and also show an apparent increase in the number of reported serogroup IIa cases from 2008 to 2015. These four Member States account for about 33% of all reported cases during this time period. The proportion of isolates in these countries serotyped during the period varied between 30 and 50% but was similar at the beginning and the end of the period.



Source: Data from The European Surveillance System – TESSy, provided by Austria, France, Germany, United Kingdom, and released by ECDC (N = 4,640).

Figure 27: Number of reported *Listeria monocytogenes* serogroup IIa cases (N = 1,679), serogroup IVb cases (N = 2,329) and cases with other serogroups (N = 632); and proportion of all cases reported with serotype/group data per year in four EU countries, 2008–2015

In summary, it is not possible to conclude whether virulence/pathogenicity has changed over the time period due to the limitations of the available data. CFRs appear not to have increased, whereas the number of serotype IIa and IVb may have increased over the time period. Only serogroups have been considered above. Typing of *L. monocytogenes* isolates is now in the transition phase from traditional methods (e.g. pulsed field gel electrophoresis, conventional/PCR-based serotyping) to sequencing (e.g. WGS) and these data are currently not available across the EU.

Table 24: Confirmed female listeriosis cases and case fatality rates by age group and year in the EU/EEA, 2008–2015

Age group (years)	2008		2009		2010		2011		2012		2013		2014		2015	
	N	CFR (%)	N	CFR (%)	N	CFR (%)	N	CFR (%)	N	CFR (%)	N	CFR	N	CFR (%)	N	CFR (%)
< 1	20	20.0	19	5.3	29	6.9	24	12.5	27	14.8	35	5.7	43	14.0	29	13.8
1–20	5	0.0	3	0.0	8	0.0	15	13.3	10	10.0	15	13.3	14	0.0	15	6.7
21–44	52	0.0	63	4.8	68	5.9	79	3.8	69	7.2	67	1.5	88	1.1	106	2.8
45–64	63	20.6	64	14.1	87	14.9	83	13.3	105	14.3	92	13.0	97	14.4	126	23.8
65–74	72	20.8	60	25.0	104	10.6	86	23.3	107	19.6	115	17.4	110	20.0	127	18.1
≥ 75	92	26.1	133	18.8	154	22.7	158	17.1	176	26.7	202	21.3	237	21.1	284	26.1
Total	**304**	**18.4**	**342**	**15.5**	**450**	**14.4**	**445**	**14.8**	**494**	**20.5**	**526**	**15.2**	**589**	**15.8**	**687**	**14.4**

Source: Data from The European Surveillance System – TESSy, provided by Austria, Belgium, Croatia, Cyprus, Czech Republic, Estonia, France, Germany, Greece, Hungary, Ireland, Latvia, Lithuania, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, United Kingdom, and released by ECDC.
CFR: case fatality rate; N: number of confirmed cases.

Table 25: Confirmed male listeriosis cases and case fatality rates by age group and year in the EU/EEA, 2008–2015

Age group (years)	2008		2009		2010		2011		2012		2013		2014		2015	
	N	CFR (%)	N	CFR (%)	N	CFR (%)	N	CFR (%)	N	CFR (%)	N	CFR (%)	N	CFR (%)	N	CFR (%)
< 1	23	13.0	27	3.7	42	19.0	25	8.0	28	21.4	15	13.3	26	15.4	27	7.4
1–20	5	0.0	5	20.0	8	12.5	7	0.0	7	14.3	8	0.0	7	0.0	7	14.3
21–44	25	20.0	18	11.1	32	12.5	23	17.4	31	6.5	27	11.1	38	7.9	35	14.3
45–64	106	19.8	122	22.1	144	21.5	142	13.4	167	16.8	166	18.7	207	15.9	189	13.8
65–74	113	23.0	140	16.4	169	16.0	143	9.8	148	16.9	172	15.7	199	14.1	218	16.1
≥ 75	110	22.7	131	18.3	228	16.7	194	19.1	206	24.8	247	17.8	305	19.7	291	23.7
Total	**382**	**20.9**	**443**	**17.6**	**623**	**17.5**	**536**	**14.2**	**587**	**19.3**	**635**	**16.9**	**782**	**16.4**	**767**	**17.5**

Source: Data from The European Surveillance System – TESSy, provided by Austria, Belgium, Croatia, Cyprus, Czech Republic, Estonia, France, Germany, Greece, Hungary, Ireland, Latvia, Lithuania, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, United Kingdom, and released by ECDC.
CFR: case fatality rate; N: number of confirmed cases.

Susceptible population

Table 26 shows the demographic changes in the EU/EEA over the time period 2009–2015. There were 514 million inhabitants in the EU/EEA in 2009; 522 million in 2015. This increase results from the increase in the elderly population (≥ 65 years old) as the younger age group (< 65 years old) has declined. In this timespan, for example, the population over 75 years old has increased from 41.6 million (or 8.1% of the population) to 47.1 million (or 9.0% of the population).

The EU is also experiencing historically low fertility rates, below the natural replacement level (an average of 2.1 children per woman in developed world economies). With fewer children being born, the relative share of young people in the EU's population has decreased. The women in the age group 25–44 years old in the EU/EEA decreased from 73 million in 2009 to 69 million in 2015. During this period the number of live births in the EU/EEA also declined from 5.5 to 5.2 million in the EU/EEA. The crude birth rate is the ratio of the number of live births during the year to the average population in that year and expressed per 1,000 inhabitants. The EU/EEA crude birth rate declined from 10.8 per 1,000 inhabitants in 2009 to 10.0 per 1,000 inhabitants in 2015.

Table 26: Evolution of the population in the EU/EEA over time, 2009–2015

Total[a]	Year						
	2009	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**
	514,946	516,168	516,110	517,357	518,616	520,554	522,221
< 75 yo[a]	473,319	473,678	472,860	473,199	473,568	474,502	475,131
≥ 75 yo[a] (%)[b]	41,628 (8.1%)	42,490 (8.2%)	43,250 (8.4%)	44,158 (8.5%)	45,048 (8.7%)	46,053 (8.8%)	47,090 (9.0%)
25–44 yo women[a] (%)[b]	73,116 (14.2%)	72,509 (14.0%)	71,828 (13.9%)	71,330 (13.8%)	70,778 (13.6%)	70,378 (13.5%)	69,941 (13.4%)
Number of live births [a] (rate[c])	5,558 (10.8)	5,558 (10.8)	5,412 (10.5)	5,378 (10.4)	5,221 (10.1)	5,280 (10.1)	5,239 (10.0)
Diabetes[a][d] (%)[b]	NA	NA	43,626 (8.45%)	NA	46,839 (9.03%)	NA	47,336 (9.06%)
Diabetes[d] in < 75 yo[a] (prev[e])	NA	NA	34,578 (7.31%)	NA	36,230 (7.65%)	NA	37,083 (7.80%)
Diabetes[d] in ≥ 75 yo[a] (prev)[f]	NA	NA	9,047 (20.92%)	NA	10,608 (23.55%)	NA	10,253 (21.77%)
Cancer (death rate)[g]			268.6	267.3	265.1	261.5	
Ischaemic heart diseases (death rate)[g]			84.91	84.32	81.79	79.65	
Chronic liver diseases (death rate)[g]			15.68	15.35	14.71	14.3	
Healthy life years[h] at 65 for females	NA	8.8	8.6	8.5	8.6	8.6	NA
Healthy life years[h] at 65 for males	NA	8.7	8.5	8.5	8.5	8.6	NA
Life expectancy at 65 for females	20.8	21.0	21.3	21.1	21.3	21.6	21.2
Life expectancy at 65 for males	17.3	17.5	17.7	17.7	17.9	18.2	17.9

NA: not available; yo: years old.
(a): Number of persons in thousand.
(b): Percentage of the total population.
(c): Crude birth rate, i.e. the ratio of the number of live births during the year to the average population in that year and expressed per 1,000 inhabitants.
(d): Type-2 only.
(e): Prevalence in < 75 yo group.
(f): Prevalence in ≥ 75 yo group.
(g): Standardised death rate by 100,000 inhabitants (death rate of a population adjusted to a standard age distribution (from http://ec.europa.eu/eurostat/tgm/table.do?tab=table&init=1&language=en&pcode=tps00116&plugin=1).
(h): The indicator 'healthy life years' at age 65 measures the number of years that a person at age 65 is still expected to live in a healthy condition.

There were only few comparable data available over time of the number of persons with underlying conditions in the EU/EEA. The percentage of persons with type-2 diabetes increased slightly during the 2011–2015 period, from 7.3% to 7.8% in the younger age group (< 75 years old) and from

20.9% to 21.8% in the elderly population (≥ 75 years old). Similarly, death rates for several serious conditions have also decreased, e.g. cancer, ischaemic heart disease, chronic liver diseases, which suggest that the proportion of people living with an underlying condition may have increased (Table 26).

The gender/age-specific prevalence (in percentage) of neoplasm (a), HIV/AIDS (b), cirrhosis and other chronic liver diseases (c), and chronic kidney disease (d), in western Europe, 1990–2015 for specific age–gender groups is presented in Figure 28. Cancer incidence is generally greater in males than in females and the incidence increases with age. This would suggest that the increase in the proportion of older people would also contribute to an increase in susceptibility. Further, for neoplasm, HIV/AIDS and cirrhosis and other chronic liver diseases, the prevalence has increased during the time period 2008–2015.

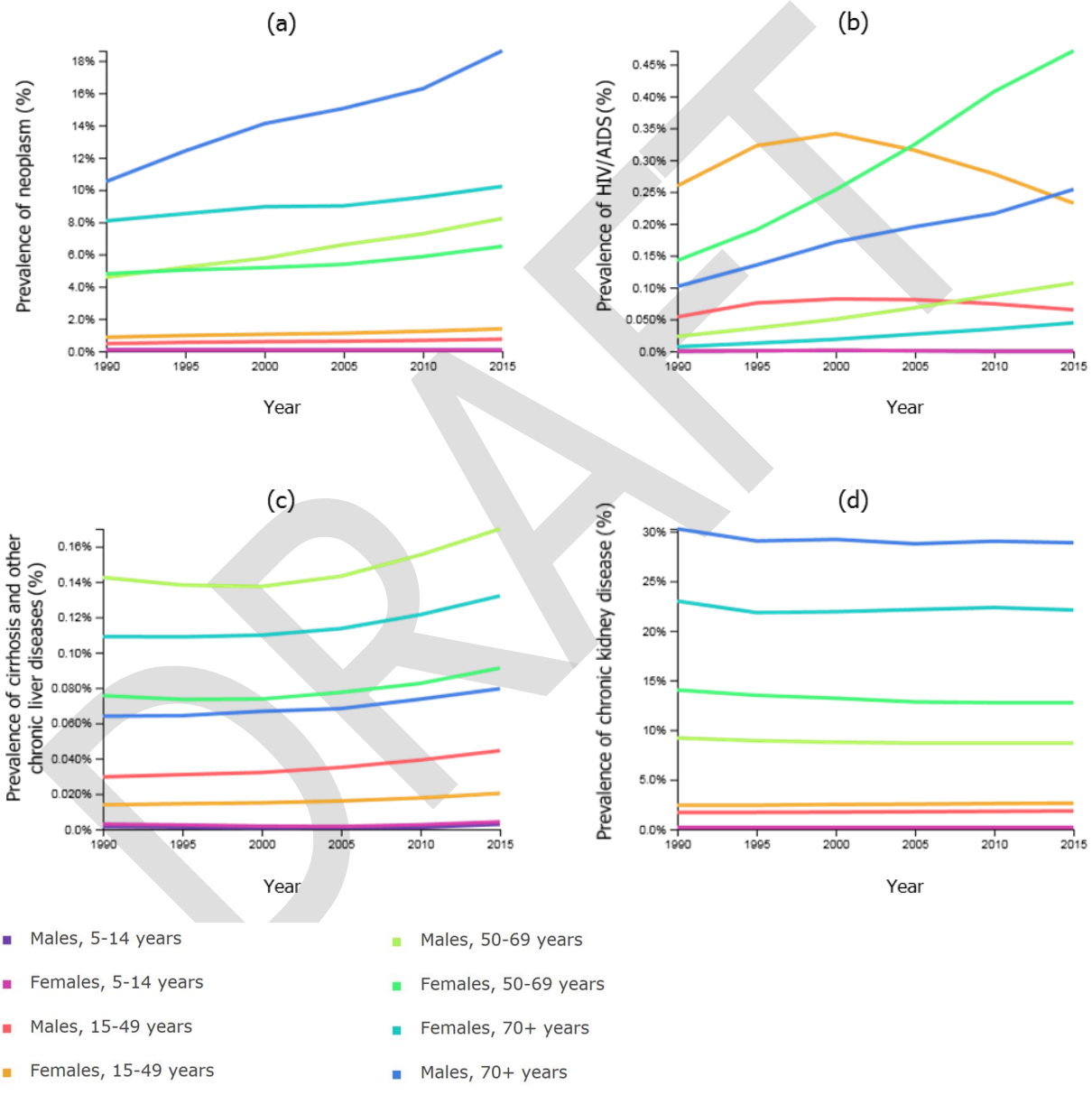


Figure 28: Prevalence (in percentage) in specific age–gender groups in western Europe, 1990–2015, of neoplasm (a), human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDS) (b), cirrhosis and other chronic liver diseases (c), and chronic kidney disease (d) (from http://www.healthdata.org/)

The number of adults (> 15 years) living with HIV in the EU/EEA was also estimated by ECDC at 810,083 in 2015 (or 0.18% of that population group). For the over 65 years age group, the figures are estimated at 3,215 for females (0.006% of that group) and 11,852 for males (0.03% of that group). For the 15–65 years age group, the figures are 216,642 for females (0.13% of that group) and 578,374 for males (0.34% of that group).

In conclusion, the increase in the number of people > 75 years, and the observations that death rates are decreasing and cancer rates increase with age, and the increase in the prevalence of several underlying conditions support the hypothesis that susceptibility has increased at least in the oldest age groups during the time period of interest.

3.6.4. Uncertainty analysis of the gQMRA model

The quantitative risk assessment model developed in this Scientific Opinion was built upon the model developed by Pérez-Rodríguez et al. (2017) in outsourcing activity 2 and in general the uncertainty sources of both models are similar. Table 28 in Appendix J presents a list and brief description of the identified sources of uncertainty. The output of the gQMRA model developed in this Scientific Opinion was the number of cases in each age–gender group and uncertainty is associated with the outcome because of the identified data and knowledge gaps. An important source of uncertainty is the dose–response relationship since it is dependent on, in addition to the epidemiological data on observed number of cases, the same data as used in the exposure assessment. The model was used to assess the distribution of cases in the baseline scenario, attribution of cases to different doses and the effect of growth, but mainly to evaluate the impact of the various factors on the reported trend of listeriosis incidence rates in the EU/EEA. The impact of uncertainty is expected to be lower for the importance analysis when the relative effects of factors were evaluated than for the absolute number predictions, since the impact is expressed as a multiplication factor, i.e. as a relative number of the number of cases in two scenarios. The uncertainty of the absolute outputs of the gQMRA model was not evaluated quantitatively but the magnitude of the uncertainties related to the factors evaluated is indicated in the importance analysis.

3.6.5. Synthesis of evidence of factors that may explain the human listeriosis trend in the EU/EEA, 2008–2015

When a listeriosis case occurs it is the unwanted outcome of an interaction between a human host and the pathogen being ingested in food. Due to data limitations listeriosis trends in humans were analysed and interpreted using age and gender as proxies for susceptible human hosts, while country variations were not considered in this Scientific Opinion. Further, only the three RTE food categories with seven subcategories included in the BLS were considered. This means that not all foods are included and that many changes in the relevant factors and in food are not considered. These include changes in the different susceptible subpopulations which may have occurred during the time period and these may have been variable in different countries. Thus, data limitations may hide trends and changes at lower levels of aggregation.

The observed trends in the TSA reflected changes in the incidence rates of human listeriosis over the time period 2008–2015 and were less than a factor of 2 for the different population groups. This corresponds to relatively small changes in terms of the absolute number of cases when considered per age group, especially in comparison with other food-borne illnesses. This makes it especially challenging to identify single or combined factors responsible for the increase in listeriosis because small changes that are difficult to detect could be behind the changes. It is also a challenge for any QMRA model to have a resolution at this level, i.e. less than 2,300 cases separated into different age and gender groups.

The TSA indicated an increasing trend for all female age groups over 25 years and for males in the ≥ 75 age groups. The increase in the female 45–64 and 65-74 age groups was borderline significant. It is assumed that the trend in females aged 25–44 years old reflects an increased incidence rate in pregnant women since more than half of the cases in this age group are known to be related to pregnancy. It is believed that this trend indicates that general changes affecting all age groups, but not changes in susceptibility, have probably occurred during the time period. The reasons for this conclusion are that listeriosis incidence rates have increased despite the fact that birth rates have decreased during the time period and that there is no reason to assume an increased susceptibility of

females but not males in the reproductive age group. Factors that are considered possible to affect all age groups include a general increase in exposure (prevalence, concentrations, and/or consumption), increased *L. monocytogenes* virulence, and/or improved surveillance. These general factors will also contribute to the observed increasing trends in the other age–gender groups, although not necessarily to the same extent. Additional factors may also be important, especially when considering the observation that the incidence rates in age groups over 45 are higher in males than in females and the difference becomes smaller in the older age groups. Below is a summary of the evidence (TSA, gQMRA, indicator data) for the contribution of different factors to the observed trends.

Factors related to the host

AQ1.1: What contribution did any change in the population size (i.e. the number) of the elderly and/or susceptible people make to the change in cases of human listeriosis in the EU/EEA in the time period 2008–2015?

This question is addressed primarily using epidemiological and population data.

An increase in the number of susceptible persons due to increased age or increased susceptibility will increase the number of human listeriosis cases by the same amount, i.e. a doubling in numbers would result in a doubling in the number of cases, everything else being equal. If only the number of persons in the susceptible groups is increased, observed incidence rates would not show an increasing trend. For this to occur, the proportions of any characteristics that affect the risk of listeriosis within the age–gender group would have to change.

Figures 29 (females) and 30 (males) show the annual listeriosis incidence (a), number of human listeriosis cases (b), and population size (c), between 2008 and 2015 for the different age groups. Dotted lines indicate the minimum or different levels of change expressed as a percentage or as a factor. The annual number of cases increases by a factor close to 1.5 for the female 45–64 age group, female and male 65–74 age groups, and by a factor close to 2 for the female and male ≥ 75 age groups and the female 25–44 age group.

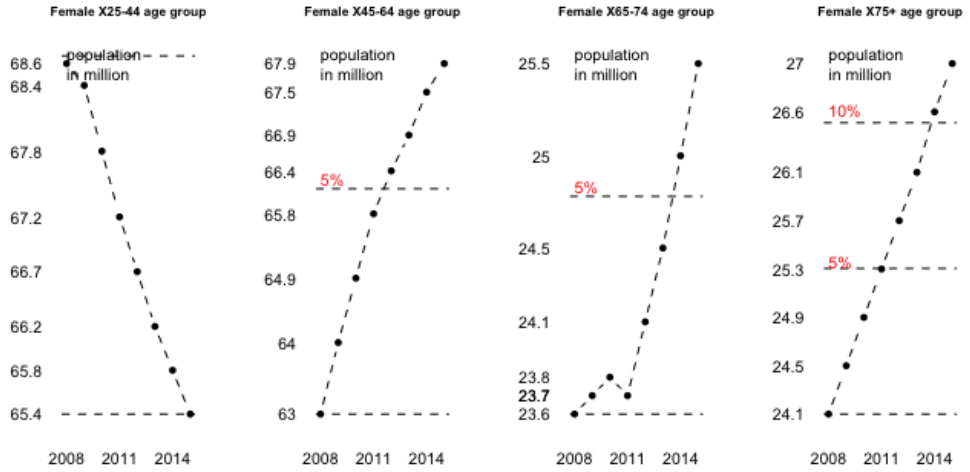
Interestingly, for the male and female 25–44 age groups the population sizes decreased by 5% (68.6 to 65.4 million for female) between 2008 and 2015. All other populations increased in size. The largest population increases are for the male 65–74 and ≥ 75 age groups where the population size increased by around 10% to 22% (Figure 30c).

If the population increase were the only factor explaining the increased number of listeriosis cases in these age groups, a population increase of more than 50% would be required instead of the observed increase of 22% (17.1/14). Moreover, for the female 25–44 age group a decrease in the population is observed.

(a)

(b)

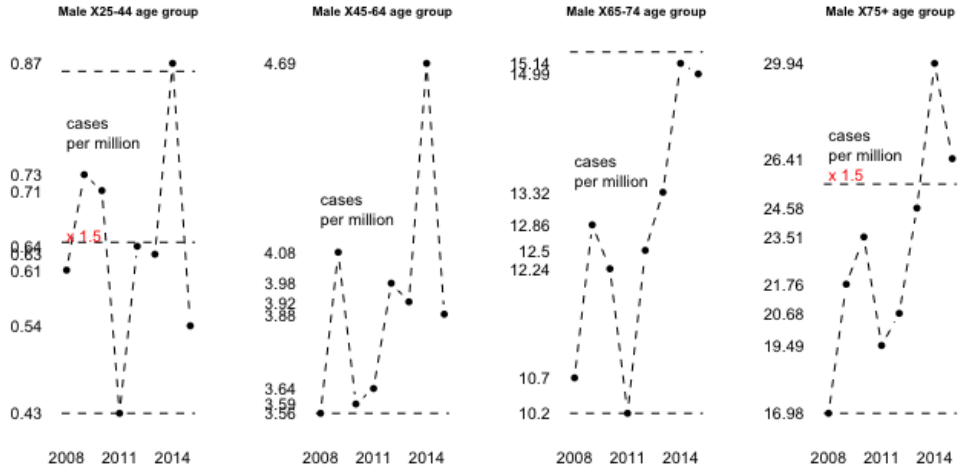
(c)



The line 1.5 shows the increase from the lowest level by a factor of 1.5, lines 5% and 10% by a percentage of 5 and 10% respectively.

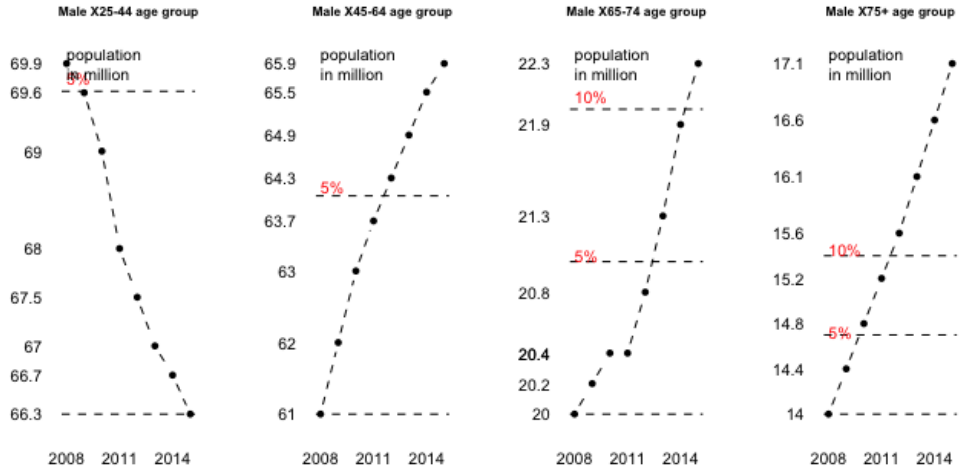
Figure 29: Annual listeriosis incidence rate (cases/million) (a), annual number of human listeriosis cases (b) and population change (c) per category of age for females

(a)



(b)

(c)



The lines 1.5 and 2 show the increase from the lowest level by a factor of 1.5, lines 5% and 10% by a percentage of 5 and 10% respectively.

Figure 30: Annual listeriosis incidence rate (cases/million) (a), annual number of human listeriosis cases (b) and population change (c) per category of age for males

AQ1.2: What contribution did any change in 'underlying condition rate' make to the change of incidence rates of listeriosis in the EU/EEA in the time period 2008–2015?

From AQ1.1 it is clear that population growth cannot explain the whole increase in the number of listeriosis cases and if the number of cases were due only to an increase in the size of these populations there would be no increasing trend in the listeriosis incidence. Thus, additional factors are needed to explain the trend. As concluded above, based on the increase in the female 25–44 group, factors affecting all age–gender groups are probably contributing to the increase. In addition, especially for the older age groups an increase in susceptibility due to underlying diseases is probably contributing to the increasing trends. The reasons for this conclusion are that indicator data show that the incidence of conditions characteristic of important risk groups, e.g. cancer cases, has increased while death rates due to these illnesses have decreased. This is expected to have resulted in an increase of the proportion of susceptible people in age groups over 44 years old which is supported by the observed increase in the prevalence of several underlying conditions. In addition, the proportion of people over 80 and 85 years old within the age group > 75 has increased and the cancer rates increase for each of these age groups. Further, support for this conclusion may be the observation that a high proportion of cases are associated with bacteraemia (Section 3.1) and as reported in Section 3.2 this symptom is typical for less virulent food-related strains and cases with one or more underlying conditions. Additional support may be the fact that the incidence of cancer as well as of listeriosis is higher in males and that the difference decreases with age. Admittedly, other differences related to gender may be as important.

Factor related to the food

AQ2.1: What contribution did any change in *L. monocytogenes* prevalence in RTE food at retail level make to the change of human listeriosis incidence rates in the EU/EEA in the time period 2008–2015?

The impact of prevalence is direct, i.e. an increase by a factor of two would increase the incidence by a factor of two (if it is assumed that the distribution for the concentration of *L. monocytogenes* remains the same).

The outcome of the gQMRA model indicates that the overall prevalence in the generic RTE food weighted to reflect consumption increases with ages over 25–44 years. This suggests that part of the increase in listeriosis incidence with age can be explained by consumption.

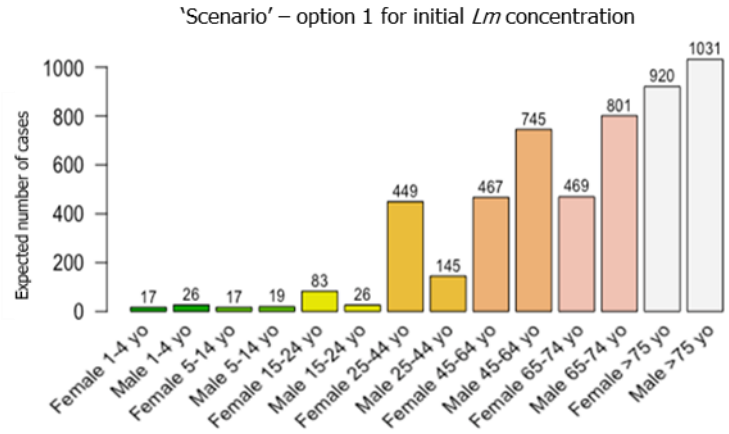
Due to data gaps and limited indicator data it is not possible to conclude to what extent an increase of prevalence with time could explain the increasing trend.

AQ2.2: What contribution did any change in *L. monocytogenes* concentration in RTE food at retail level make to the change of human listeriosis incidence rates in the EU/EEA in the time period 2008–2015?

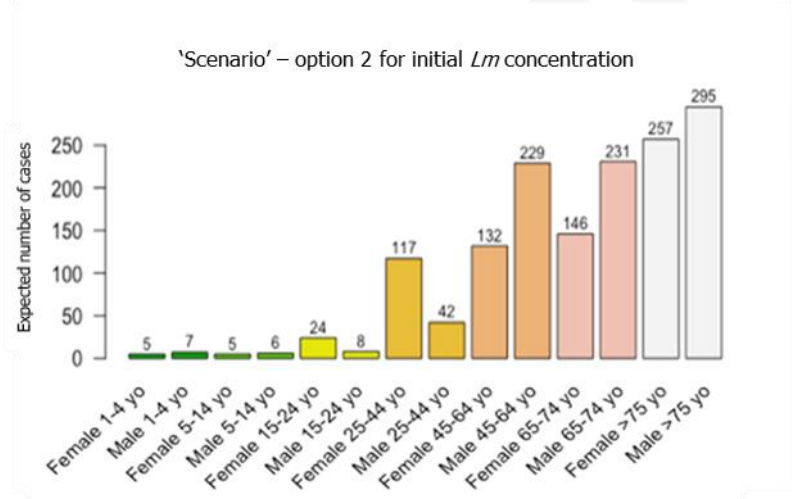
As shown in the importance analysis, the gQMRA model is very sensitive to the maximum population density (MPD) and small changes may result in a multiplication of risk by a factor of 2. The impact of initial concentration is also shown in Figure 31 where the estimated number of human listeriosis cases using the three different options described in the methodology section is presented.

The option using the BLS data resulted in a substantially larger number of human listeriosis cases than the baseline and option 2 (Figure 31). Thus, the concentration at retail and the MPD have a large impact on the listeriosis risk. Some indicator data suggest that a large number of servings exists on the market within a dose range that, according to the gQMRA model, explains more than 90% of listeriosis cases, i.e. over 3 $\log_{10}$ CFU/g. In contrast, there are limited data to determine the extent to which shifts in concentration, either in non-compliant foods, MPD or the concentration at retail, have contributed to the increased listeriosis trend. The indicator data, i.e. the RASFF data, were variable but did not indicate any consistent increase in either the mean or the maximum concentrations. At the same time these data are very limited and it is therefore uncertain to what extent it reflects the real situation.

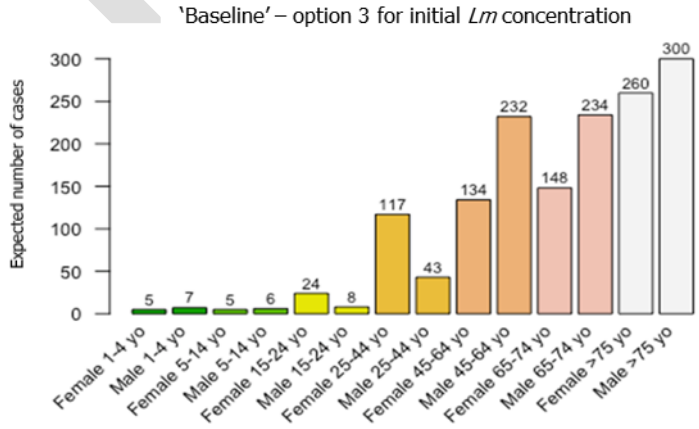
(a)



(b)



(c)



(a) Option 1: using only the distributions estimated with BLS data; (b) option 2: using only the distributions estimated with US data (Gombas et al., 2003); and (c) option 3: using fish distribution from BLS data, and meat and cheese distributions from US data (Gombas et al., 2003).

Figure 31: Expected number of human listeriosis cases per subpopulation and per year using three options for the initial concentration of *L. monocytogenes* in the seven RTE food subcategories (1 million iterations)

AQ2.3: What contribution did any change in storage conditions (temperature, time) after retail (i.e. consumer phase) make to the change of human listeriosis incidence rates in the EU/EEA in the time period 2008–2015?

The gQMRA indicated that the potential for an impact on the human listeriosis incidence was quite large, especially for the storage time. In addition, the summary of the literature on food handling (including storage times and temperatures) indicated that the proportion of unsafe behaviours in risk groups is large, and sometimes related to age or socioeconomic factors. This supports the notion that storage conditions contribute to the human listeriosis incidence. Different combinations of maximum remaining storage time and mode of storage time may lead to a multiplication by a factor of 2. Several trends in society, for instance in relation to sustainability and efforts to decrease food waste or a weak economy, may be hypothesised to influence changes in these parameters. Similarly, lack of temperature control among different consumer groups has also been reported. Due to data gaps it is not possible to conclude that consumer storage conditions (times, temperatures) have changed during the time period and contributed to the increasing human listeriosis trends.

AQ2.4: What contribution did any change in consumption (serving size and frequency) make to the change of human listeriosis incidence rates in the EU/EEA in the time period 2008–2015?

As can be seen in Table 22 (results baseline model, total number of eating occasions per year (TEO)), the impact of the number of servings is direct, i.e. an increase of serving frequencies by a factor of two would increase the number of human listeriosis cases per year by a factor of two. The same is true for the serving size.

There is some support in the indicator data for an increase in the consumption frequency of RTE foods, e.g. cooked RTE foods and smoked salmon, but this is based on limited data.

Results from the gQMRA model indicated that differences in consumption among the age groups influenced the probability of exposure to *L. monocytogenes* through the effect on the prevalence. Due to data gaps it is not possible to conclude whether serving sizes or the number of eating occasions have increased during the time period or to what extent it might have contributed to the increased trend of human listeriosis.

Factors related to the surveillance system

AQ3.1: What contribution did any change of (improved) surveillance make to the change of human listeriosis incidence rates in the EU/EEA in the time period 2008–2015?

The impact of this factor is direct, i.e. an improvement of surveillance by a factor of two would increase the human listeriosis incidence by a factor of two. Estimations of under-reporting and under-ascertainment of listeriosis in Canada, the USA and the UK have resulted in factors of around 1.7 to 2 (Mead et al., 1999; Adak et al., 2002; Thomas et al.) which are in the same range as the largest increases in listeriosis trends. The indicator data show that changes in the surveillance in some countries may have contributed to an increase in the number of reported cases during the time period. It is not possible to draw conclusions on the quantitative impact of this on the observed trend.

Factors related to the bacterium

AQ4.1: What contribution did any change in virulence make to the change of human listeriosis incidence rates in the group of interest in the EU/EEA in the time period 2008–2015?

The available indicator data were limited and the analysis could only be based on serogroups and mortality rates. These data did not indicate an increase in the virulence/pathogenicity.

Based on the indicator data it is not possible to conclude that the virulence of *L. monocytogenes* has increased during the period. With new data becoming available it should be possible to evaluate this factor more appropriately.

3.6.6. Conclusions of factors contributing to the human listeriosis trend in the EU/EEA, 2008–2015

Summarising remarks based on the gQMRA model and the baseline scenario:

- The frequency of exposure (i.e. the prevalence of *L. monocytogenes* in RTE foods) appears to increase with increasing age over 25 years old for both genders, due to differences in consumption patterns.
- Based on predictions of the gQMRA model, the expected number of human listeriosis cases per year is reduced by 37% (from 1,523 to 953) in the absence of growth from retail onwards.
- Based on the gQMRA model and empirical data on initial *L. monocytogenes* concentrations reflecting contaminated RTE food on the market (of the considered foods 'RTE fish,' 'RTE meat' and 'RTE cheese,' according to specifications from the BLS), 92% of listeriosis cases for all subpopulations are attributable to doses above 10^5 CFU per serving. Assuming an average serving size of 50 g, this would correspond to an average *L. monocytogenes* concentration in RTE foods of 2,000 CFU/g at the time of consumption.

Factors that may have impacted on the trend of human listeriosis **cases/incidence rates** in the EU/EEA during 2008–2015 were classified based on the quality of the available evidence applying the probability scales as defined in the draft EFSA guidance on uncertainty (EFSA Scientific Committee, 2016):

- **Class 1:** factors **likely** (66–90%) to have contributed to the trend (based on the potential impact when changing the factor according to modelling or other information, and support from indicator data and expert opinion);
- **Class 2:** factors that **as likely as not** (33–66%) have contributed to the trend (based on expert opinion due to the potential impact when changing the factor according to modelling or other information, but with no or limited empirical evidence to support the conclusion); and
- **Class 3:** factors that are **inconclusive** and therefore may or may not have contributed to the trend (based on expert opinion due to the potential impact when changing the factor according to modelling, but no or limited empirical evidence).

The following factors were considered to belong to **class 1**:

- For the increased number of human listeriosis cases in the EU/EEA
  - An increased **population size of the elderly and susceptible population** (except in the 25–44 female age group which has decreased).
- For the increased incidence rates/cases of human listeriosis in the EU/EEA
  - An increased **proportion of susceptible persons** in age groups over 45 years of both genders. The increasing trend in the female 25–44 age group (pregnancy-related) suggests that a factor other than susceptibility must have contributed since susceptibility is not expected to have changed in this population during the time period. The additional factor may be any of those evaluated and would likely contribute to the trend in all age groups but possibly to a varying degree.

The following factors were considered to belong to **class 2**:

- For the increased incidence rates/cases of human listeriosis in the EU/EEA
  - An increased consumption (number of servings per person) of RTE foods in the EU/EEA
  - An improved surveillance of human listeriosis in the EU/EEA.

The following factors were considered to belong to **class 3**:

- For the increased incidence rates of human listeriosis in the EU/EEA

- *L. monocytogenes* concentration in the three considered RTE food categories[37] at retail
- *L. monocytogenes* prevalence in the three considered RTE food categories at retail
- *L. monocytogenes* virulence potential
- Storage conditions (time and temperature) after retail of the three considered RTE food categories.

Several data gaps limited the evaluation of factors behind the observed listeriosis trend and contributed to uncertainties in the assessment outcome. Data gaps include harmonised data collected using a sampling strategy suitable for surveillance over time on:

- prevalence and concentration of *L. monocytogenes* in RTE foods
- consumption of RTE foods
- prevalence of risk groups by age and gender
- retail and home storage temperatures
- *L. monocytogenes* virulence.

4. Conclusions

ToR 1 To summarise and critically evaluate the most recent information on *L. monocytogenes* in RTE foods, and in particular from the following sources: (a) EU-wide baseline survey and monitoring data and (b) the three EFSA outsourcing activities

- Despite an increase in confirmed listeriosis cases during the time period 2008–2015 and considering that the under-reporting/under-ascertainment of human invasive listeriosis is low compared to many other food-borne pathogens, fewer than 2,300 cases per year were reported in the EU/EEA.
- Notification rates (i.e. notified incidence rates) of invasive listeriosis in the EU/EEA generally increase with increasing age, with the highest incidence rates observed in the age groups over 65 years old and in children below 1 year of age (mainly pregnancy-related cases).
- In addition to age/susceptibility, medical practices for other ailments have been hypothesised to be risk factors for human listeriosis. Treatments with PPI, for example, which increase gastric pH, can increase susceptibility to listeriosis.
- During the 2008–2015 time period, bloodstream infections were the most commonly reported clinical forms of invasive *L. monocytogenes* infections (71.8% of confirmed cases), followed by meningitis (19.4% cerebrospinal fluid samples). The overall annual CFR ranged from 12.7 to 20.5%.
- There is ample evidence for a high variability regarding the virulence potential and pathogenicity of different *L. monocytogenes* isolates. Epidemiological data combined with genetic sequence information and results from animal models (based on more than 6,000 isolates from clinical specimens and food items) indicate that only 12 CC make up almost 80% of all isolates, and that different levels of virulence may be associated with these.
- Listeriosis is a food-borne illness, but CCs have, according to one study, been termed 'infection-associated,' 'food-associated' or `intermediate´ depending on the relative proportion of isolates from clinical cases, food or both. Uncertainty may be associated with this classification due to knowledge gaps about factors influencing the isolation and detectability of different strains from different matrices. Several factors related to isolation bias during enrichment and detection procedures have been reported, e.g. composition of detection media, natural microbiota in the sample, intra-species competition mediated by bacteriocins, bacteriophages or cell-cell contact.

[37] 'RTE fish,' 'RTE meat' and 'RTE fish,' according to specifications from the EU-wide baseline survey.

- 'Infection-associated' CCs are most commonly associated with CNS and MN infections as opposed to bacteraemia alone. 'Food-associated' CCs are rarely isolated from clinical samples but, when recovered from clinical specimens, usually isolated from blood.
- 'Food-associated' CCs are more frequently associated with highly immunocompromised patients or patients showing a higher number of severe comorbidities. Based on humanised mouse models it appears that these predominately 'food-associated' CCs are less invasive (hypovirulent) than the 'infection-associated' CCs.
- Despite the observed variability in their virulence potential, any *L. monocytogenes* strain has the ability to result in severe human listeriosis because of the complex interaction between the pathogen, food and host.
- Most listeriosis cases appear to be sporadic and reported outbreaks (strong-evidence outbreaks accounting for less than 4% of cases during the time period) are commonly small. This makes it difficult to detect links between human cases and causative foods. Next generation sequencing (NGS) techniques, when combined with epidemiological information, may have the potential to improve this detection.
- Results from the outsourced study to attribute human cases to different animal sources are limited not only by the representativeness of isolates from all relevant sources but also by difficulties with identifying their origin, since contamination during processing is so important.
- Persistence of *L. monocytogenes* in food processing environments is considered to be the major source of RTE food contamination. Persistence appears to be the result both of improper hygiene conditions and the highly adaptive capacity of these bacteria against physical–chemical factors, for example, biofilm-forming capacity, but the relative role of each is unclear.
- RTE food categories typically associated with human listeriosis, i.e. 'meat and meat products,' 'fish and fish products,' and 'milk and milk products' continue to have a significant public health impact. In addition, food of plant-derived origin or even frozen foods have been implicated in outbreaks (e.g. cantaloupe, caramel apples, ice cream) illustrating that almost all RTE foods under certain unexpected conditions may support growth and/or that when consumed by highly susceptible people, have the potential to contribute to the burden of disease.
- In the time period 2008–2015, the annual reported non-compliance of *L. monocytogenes* in RTE foods at processing was highest in 'RTE fishery products' (3–10%), followed by 'RTE products of meat origin other than fermented sausage' (1–7%). Non-compliance in the remaining RTE food subcategories was 2% or less. At retail the annual reported non-compliance was generally lower than at processing and was below 1% for most years. The lower level of non-compliance at retail is at least partly explained by the application of different limits of FSC at retail and processing.
- According to the EU-wide BLS (2010–2011), *L. monocytogenes* was more prevalent in RTE smoked and gravad fish (10.3%; 1.7% above 100 CFU/g), than in RTE heat-treated meat (2.1%; 0.43% above 100 CFU/g) and RTE soft and semi-soft cheese (0.5%; 0.1% above 100 CFU/g) at the end of their shelf life.
- Cooked meat and heat-treated sausages were the RTE food subcategories with most consumed servings per person and per year in the EU/EEA and for meat products the number of servings was in general greater for males than for females.
- Combining the BLS and consumption data indicates that approximately 55 million servings of RTE meat and meat products contaminated with more than 100 CFU/g may be consumed per year by the population over 75 years old in the EU/EEA.
- Unsafe practices (including storage time and temperatures) are not uncommon within the elderly group (> 10% of persons studied), and have a potential impact on the human listeriosis risk. There is a wide variation within the broadly defined consumer groups and it is thus problematic to generalise about the food handling behaviours of these groups and on how this may contribute to trends of human listeriosis.

- Temperature of domestic refrigerators is highly variable. A review of 23 available survey studies from 1991 to 2016 showed mean, minimum and maximum temperatures ranging from < 5 to 8.1°C, -7.9 to 3.8°C and 11.4 to 20.7°C, respectively.
- The extent of different behaviours among risk groups between EU Member States may vary to the same extent that socioeconomic factors, traditions and types of food vary. Since the majority of studies of food handling are from a few countries only, this may lead to some uncertainty about the generalisability of the results presented.
- The average probability of a single *L. monocytogenes* CFU to cause illness in a specific host (the *r* value), reflects the strain virulence and host susceptibility, and ranges three orders of magnitude, from the least to the most susceptible subpopulations. Reported *r* values for specific outbreaks with highly susceptible populations increase the range by another five orders of magnitude. This means that the probability of a single CFU to cause illness may range 100 million times depending on variability in host susceptibility and *L. monocytogenes* virulence.
- A lognormal-Poisson extension of the exponential dose–response model, incorporating the virulence and susceptibility variability for 11 population groups, suggests that most listeriosis cases are linked to the ingestion of food contaminated with medium to high (3.5 – 7.5 $\log_{10}$ CFU/serving) concentrations of *L. monocytogenes*.
- Most risk characterisations consider three risk populations (i.e. pregnant women/perinatals, the elderly (> 60 or > 65 years old), and the intermediate population that do not belong to either of these categories) and have not addressed gender differences. This limitation can be addressed with dose–response data and other input data developed at a finer resolution in some recent publications and in the present Opinion.
- Based on the quantitative risk characterisation of *L. monocytogenes* in various RTE food categories (heat-treated meat; smoked and gravad fish; and soft and semi-soft cheese) in the EU:
  - The most cases were predicted in the elderly population (≥ 65 years old) (48% of cases) followed by the pregnant population (41% of cases) and the healthy population < 65 years (11% of cases).
  - The food subcategory associated with the largest number of cases per year was cooked meat (863 cases). After that followed sausage (541 cases), gravad fish (370 cases), cold-smoked fish (358 cases), pâté (158 cases), soft and semi-soft cheese (19 cases) and hot-smoked fish (7 cases).
  - Estimated risks expressed as the median number of cases per million servings was in general highest for the pregnant population, followed by the elderly and last the healthy (< 65 years) population.
  - Uncertainty sources for some variables such as initial prevalence of *L. monocytogenes* in RTE foods should be further elucidated as well as variability in *L. monocytogenes* growth when types of product and populations are compared.

ToR 2 To discuss and evaluate the factors related to contamination in the food chain and the consumption patterns that may contribute to the reported trend of listeriosis incidence in the EU

- For the time period 2008–2015 TSA of 14,002 confirmed human listeriosis cases in the EU/EEA was carried out at different levels of aggregation, e.g. aggregated by total confirmed cases, and disaggregated by 14 age–gender groups.
- The aggregated TSA did not show an increasing trend of listeriosis incidence rates in the EU/EEA. This is partly a consequence of the presence of changing dynamics, autocorrelation and strong seasonality in the aggregated analysis. This is in contrast with the disaggregated analyses for which clear trends were shown and where some of the aforementioned characteristics were present to a lesser extent.

- For females, the incidence rate of confirmed human listeriosis significantly increased for the 25-44 and ≥ 75 age groups in this time period with a monthly increase estimated at 0.64% and 0.70%, respectively. For the female 45-64 and 65-74 age groups the increasing trend was borderline significant with a monthly increase estimated at 0.43% and 0.30%, respectively.
- For males, the incidence rate of confirmed human listeriosis cases increased significantly for the ≥ 75 age group only with a monthly increase of the incidence rate estimated at 0.50%.
- In 2015, the listeriosis incidence rate was higher for males than for females in the age groups over 45 years old. The opposite was true for the female 15-24 and 25–44 age groups believed to largely reflect pregnancy-related listeriosis.
- The highest incidence rate in the EU/EEA in the period 2008–2015 is seen in the ≥ 75 age group resulting in 2015 in incidence rates of 2.20 and 1.30 cases per month per million persons for males and females respectively. All other age and gender groups have lower incidence rates.
- There are several sources of uncertainty, which can lead to under- or overestimation of the observed trends. Due to the available data, the analysis and understanding of trends were performed using age and gender as proxies for susceptible populations and not including countries as a covariate. This is a limitation and means that the observed trends may hide trends among subgroups or be true for only a subset of the age–gender–country population.
- Based on the *Listeria monocytogenes* gQMRA model and empirical data on initial *L. monocytogenes* concentrations reflecting contaminated RTE food on the market (of the considered foods 'RTE smoked and gravad fish,' 'RTE heat-treated meat' and 'RTE soft and semi-soft cheese,' according to specifications from BLS), 92% of listeriosis cases for all subpopulations are attributable to doses above 10^5 CFU per serving. Assuming an average serving size of 50 g, this would correspond to an average *L. monocytogenes* concentration in RTE foods of 2,000 CFU/g at the time of consumption.
- Based on predictions of the gQMRA model, the expected number of human listeriosis cases per year is reduced by 37% (from 1,523 to 953) in the absence of growth from retail onwards.
- The frequency of exposure (i.e. the prevalence of *L. monocytogenes* in RTE food) appears to increase with increasing age over 25 years old for both genders, due to differences in consumption patterns.
- Factors that may have contributed to the increasing trend of human listeriosis cases/incidence rates in the EU/EEA during 2008–2015 were classified, based on the potential impact when changing the factor according to modelling or other information, the degree of support from indicator data, and expert opinion, into probability scales as defined in the draft EFSA guidance on uncertainty (EFSA Scientific Committee, 2016).
- Factors considered as **likely** (66–90%) were:
  - An increased **proportion of susceptible persons** in age groups over 45 years for both genders. The increasing trend in the female 25–44 age group (mainly pregnancy-related) suggests that a factor other than susceptibility must have contributed since susceptibility is not expected to have changed in this population during the time period. The additional factor may be any of those evaluated and would likely contribute to the trend in all age groups but possibly to a varying degree.
  - An increased **population size of the elderly and susceptible population** (except for the 25–44 female age group which has decreased). This factor would only contribute to the number of listeriosis cases but not the increase in incidence rates.
- Factors considered **as likely as not** (33–66%) were:
  - an increased **consumption** (number of servings per person) of RTE foods in the EU/EEA;

- an improved **surveillance** of human listeriosis in the EU/EEA.

- Inconclusive factors were:
  - *L. monocytogenes* **concentration** in the three considered RTE food categories at retail;
  - *L. monocytogenes* **prevalence** in the three considered RTE food categories at retail;
  - *L. monocytogenes* **virulence** potential;
  - **storage conditions** (time and temperature) after retail of the three considered RTE food categories.
- Due to data limitations the present evaluation was based on only three RTE categories which is a limitation of the assessment.
- Uncertainty is associated with the gQMRA model because of data and knowledge gaps. An important source of uncertainty is the dose–response relationship since it is dependent on the same data as used in the exposure assessment and the epidemiological data. However, the impact of uncertainty is expected to be lower for the importance analysis when the relative effects of factors were evaluated than for the absolute number predictions.
- Data gaps include representative data collected across the EU/EEA using a harmonised sampling strategy suitable for surveillance over time on:
  - prevalence and concentration of *L. monocytogenes* in RTE foods;
  - consumption of RTE foods;
  - prevalence of different risk groups by age and gender;
  - retail and home storage temperatures; and
  - *L. monocytogenes* virulence.

5. Recommendations

- To raise the awareness of all stakeholders in the food chain about the potentially increasing problem of *L. monocytogenes* in RTE foods since the proportion of European citizens in high-risk groups is expected to increase in the EU/EEA.
- To implement innovative programmes to generate data on *L. monocytogenes* in food that are comparable across Member States and time in the EU as existing monitoring has other objectives and is not appropriate for evaluating trends over time.
- To address the need for data to evaluate changes in consumption of RTE foods, and other food categories over time in the EU.
- To improve the information for risk assessment and risk management by collecting comparable data on human listeriosis cases that are more aligned with the concept of risk groups (e.g. pregnancy, different types of cancer, renal or liver failure) in terms of the number of cases within these groups and their consumption habits (today based only on age and gender) as well as socioeconomic–demographic data.
- To obtain better information on how the dietary practices and food handling of elderly groups are affected by ageing and how this may be linked to an increased exposure to *L. monocytogenes*.

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5387 # Abbreviations

AIDS	acquired immune deficiency syndrome
AP	antimicrobial preservatives
AQ	assessment question
AR	acidity regulators
ARIMA	autoregressive integrated moving average
ATR	acid tolerance response
a_w	water activity
BIOHAZ Panel	EFSA Panel on Biological Hazards
BLS	EU-wide baseline survey
CC	clonal complex
CDF	cumulative distribution function
CFR	case fatality rate
CFU	colony forming units
CI	confidence interval
CNS	central nervous system
CPM	cardinal parameter models
CSF	cerebrospinal fluid
DLM	dynamic linear model
DR	dose reponse
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
ECDF	empirical cumulative distribution function
EFSA	European Food Safety Authority
Eurostat	The Statistical Office of the European Union
FAO	The Food and Agriculture Organization of the United Nations
FPE	food processing environment
FSC	food safety criteria
GAD	glutamate decarboxylase
GHP	good hygiene practices
gQMRA	*Listeria monocytogenes* generic QMRA
HACCP	hazard analysis and critical control points
HIV	human immunodeficiency virus
InlA	Internalin A
IRTA	Institut de Recerca i Tecnologia Agroalimentàries
MIC	minimum inhibitory concentration
MN	maternal–neonatal
NGS	next generation sequencing

OR	odds ratio
PAR	Poisson autoregressive model
PPI	proton pump inhibitors
QMRA	quantitative microbiological risk assessment
RASFF	EU Rapid Alert System for Food and Feed
ROP	reduced oxygen packaging
RTE	ready-to-eat
SNP	single nucleotide polymorphism
TESSy	The European Surveillance System
ToR	terms of reference
TSA	time series analysis
UCO	University of Cordoba
WGS	whole genome sequencing
WHO	World Health Organization

5388

Appendix A – Food safety criteria (FSC) for *Listeria monocytogenes* in ready-to-eat (RTE) foods

5389 Commission Regulation (EC) No 2073/2005[38] on microbiological criteria for foodstuffs lays down food
5390 safety criteria (FSC) for *L. monocytogenes* in ready-to-eat (RTE) foods. This Regulation came into
5391 force in January 2006.

Food category	Micro-organisms/their toxins, metabolites	Sampling-plan (1)		Limits (2)		Analytical reference method (3)	Stage where the criterion applies
		n	c	m	M		
1.1. Ready-to-eat foods intended for infants and ready-to-eat foods for special medical purposes (4)	*Listeria monocytogenes*	10	0	Absence in 25 g		EN/ISO 11290-1	Products placed on the market during their shelf-life
1.2. Ready-to-eat foods able to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes	*Listeria monocytogenes*	5	0	100 cfu/g (5)		EN/ISO 11290-2 (6)	Products placed on the market during their shelf-life
		5	0	Absence in 25 g (7)		EN/ISO 11290-1	Before the food has left the immediate control of the food business operator, who has produced it
1.3. Ready-to-eat foods unable to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes (4) (8)	*Listeria monocytogenes*	5	0	100 cfu/g		EN/ISO 11290-2 (6)	Products placed on the market during their shelf-life

5392

(1) n = number of units comprising the sample; c = number of sample units giving values over m or between m and M.
(2) For points 1.1-1.24 m=M.
(3) The most recent edition of the standard shall be used.
(4) Regular testing against the criterion is not useful in normal circumstances for the following ready-to-eat foods:
— those which have received heat treatment or other processing effective to eliminate *L. monocytogenes*, when recontamination is not possible after this treatment (e.g. products heat treated in their final package),
— fresh, uncut and unprocessed vegetables and fruits, excluding sprouted seeds,
— bread, biscuits and similar products,
— bottled or packed waters, soft drinks, beer, cider, wine, spirits and similar products,
— sugar, honey and confectionery, including cocoa and chocolate products,
— live bivalve molluscs.
(5) This criterion applies if the manufacturer is able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit 100 cfu/g throughout the shelf-life. The operator may fix intermediate limits during the process that should be low enough to guarantee that the limit of 100 cfu/g is not exceeded at the end of the shelf-life.
(6) 1 ml of inoculum is plated on a Petri dish of 140 mm diameter or on three Petri dishes of 90 mm diameter.
(7) This criterion applies to products before they have left the immediate control of the producing food business operator, when he is not able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 cfu/g throughout the shelf-life.
(8) Products with pH ≤ 4,4 or a_w ≤ 0,92, products with pH ≤ 5,0 and a_w ≤ 0,94, products with a shelf-life of less than five days are automatically considered to belong to this category. Other categories of products can also belong to this category, subject to scientific justification.

5393

5394 Food business operators (FBOs) shall ensure that foodstuffs comply with these microbiological
5395 criteria. To this end the food business operators at each stage of food production, processing and
5396 distribution, including retail, shall take measures, as part of their procedures based on Hazard
5397 Analysis and Critical Control Point (HACCP) principles together with the implementation of good
5398 hygiene practice, to ensure the following:

5399 • that the supply, handling and processing of raw materials and foodstuffs under their control
5400 are carried out in such a way that the process hygiene criteria are met;

5401 • that the food safety criteria applicable throughout the shelf life of the products can be met
5402 under reasonably foreseeable conditions of distribution, storage and use.

5403 As necessary, the FBOs responsible for the manufacture of the product shall conduct studies to
5404 investigate compliance with the criteria throughout the shelf life. In particular, this applies to the RTE
5405 foods that are able to support the growth of *L. monocytogenes* and that may pose a
5406 *L. monocytogenes* risk for public health.

5407 In this Regulation RTE food is defined as 'Food intended by the producer or the manufacturer for
5408 direct human consumption without the need for cooking or other processing effective to eliminate or
5409 reduce to acceptable level microorganisms of concern.´

5410

[38] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, 26 pp.

Appendix B – Additional information of the time series analysis (TSA)

The R-code of the *Listeria monocytogenes* time series analysis (TSA) model is included below as well as the inputs of the model (Tables 27-29). The latter are included in an Excel files with tables. Only the headings are shown hereunder with some hypothetical values to clarify the content of the table.

Table 27: Data used for conducting the aggregated time series analysis (TSA) (read in as "totals.csv" in R)

Year	Month	NumberOfCases[(a)]	pop[(a)]
2008	1	22	44.2×10^7
2008	2	…	…
2008	…	…	…
2008	12	15	44.2×10^7
…	…	…	…
2015	12	5	44.5×10^7

NumberOfCases: number of *Listeria monocytogenes* cases in a specific month and year; pop: total population in a specific month and year.
(a): Values shown hypothetical and for illustrative purposes only.

Table 28: Human listeriosis data used for conducting the disaggregated age-gender groups time series analysis (TSA) (read in as "merged_eu.csv" in R)

AgeGroup_ECDC	Gender	Month	Year	NumberOfCases[(a)]	pop[(a)]	date
X01-04	Female	1	2008	0	25×10^5	01-01-08
X01-04	Male	1	2008	0	26×10^5	01-01-08
X05-14	Female	1	2008	0	97×10^5	01-01-08
X05-14	Male	1	2008	2	102×10^5	01-01-08
X15-24	Female	1	2008	0	249×10^5	01-01-08
…	…	…	…	…	…	…
X75+	Female	12	2015	27	255×10^5	01-12-15
X75+	Male	12	2015	49	223×10^5	01-12-15

NumberOfCases: number of *Listeria monocytogenes* cases in a specific month and year; pop: total population in a specific month and year.
(a): Values shown hypothetical and for illustrative purposes only.

Table 29: Population data used for conducting the disaggregated age-gender groups time series analysis (TSA) (read in as "merged_eu_wide.csv" in R)

date	X01.04.Female.cases[(a)]	…	X75..Male.cases[(a)]	X01.04.Female.pop[(a)]	…	X75..Male.pop[(a)]
01-01-08	0	…	4	25×10^5	…	20×10^6
01-02-08	1	…	7	25×10^5	…	20×10^6
01-03-08	2	…	2	25×10^5	…	20×10^6
…	…	…	…	…	…	…
01-12-15	2	…	5	24×10^5	…	23×10^6

Xxx.xx.Female/Male.cases: number of *Listeria monocytogenes* female/male cases in a specific age group xx.xx during a specific month in a specific year (month-year as indicated in "date"). Xxx.xx.Female/Male.pop: total female/male population in a specific age group xx.xx at a specific month in a specific year (month-year as indicated in "date").
(a): Values shown hypothetical and for illustrative purposes only.

```
################################
################################
# Part 1: Aggregated analysis
################################
################################
remove(list=ls())
# Read in the data (this dataset was created from the initial dataset)
tmp <- read.csv("totals.csv", header=T, sep = ";")
L <- ts(tmp[,3], start=c(2008,1), freq=12)
L2 <- ts(10000000*tmp[,3]/tmp[,4], start=c(2008,1), freq=12)
# Check the total number of cases that should be 14002
sum(L)
# Source in the pests.r code for the PAR models
# Can be found in http://www.utdallas.edu/~pbrandt/code/pests.r
source("pests.r")
# Plot the data and check the AR dynamics: Figure in the report
par(mfcol=c(2,1), mai=c(0.9,1.1,0.3,0.15))
plot(L, main="", ylab="Cases", xlab="")
plot(L2, main="", ylab="Cases/10000000")
counts.acf(L, main="", ylab="Correlations")
# Very seasonal set of correlations, so PAR(p) is not going to work
# and these are not really rare counts that would follow a Poisson process
# Non-PAR models tried here too.
# Arima models checked but Seasonal part process that is non-stationary,
# since the seasonal AR ~ 1 and the seasonal MA > |0.5|
# Consider a non-parametric state-space decomposition: Figure not in the report
plot(stl(L, s.window=13))
# Seems to have strong, maybe changing trend and a well-defined seasonal
# A DLM seems appropriate
# Load the library / package before executing the following line.
library(dlm)
level0 <- L[1]
# This builds a random walk walk model with dummy seasonals
# A second order model is tried further but the second order trend is not
# significant.
buildm <- function(u) {
  # Start with a random walk model
  # We will estimate the state-space variance here as
  # args u[1]
  trend <- dlmModPoly(order=1, dV = 1e-7, dW = exp(u[1]),
                      m0 = c(level0),
                      C0 = 1)
  # Now add deterministic seasonal dummies and estimate the
  # variation around these as exp(u[2])
  seas <- dlmModSeas(freq=12, dV=exp(u[2]))  # det. seasonal
  return(trend + seas)
}
# Initialize the model
init <- rep(0,2)
# Estimate with MLE
outMLE <- dlmMLE(L, init, buildm, hessian=TRUE)
# Look at the MLE output and the standard errors
# following gives V and W reported in the Opinion
print(exp(outMLE$par))
sqrt(diag(solve(outMLE$hessian)))
dlmL <- buildm(outMLE$par)
# Inspect the parameters
# Variances of the state equation
sqrt(diag(W(dlmL))[c(1:2)])
# Now generate the in-sample filtered and smoothed DLMS
LFilter <- dlmFilter(L, dlmL)
LSmooth <- dlmSmooth(LFilter)
seFilter <- residuals(LFilter, sd=TRUE)
# Plot out the data, filtered and smoothed values - Figure in the Opinion.
par(mfrow=c(3,1), mai=c(0.8,0.5,0.5,0.15), cex.axis=1.5, cex.lab=1.5)
plot(window(cbind(L,dropFirst(LFilter$m[,1]),
           dropFirst(LFilter$m[,1]) + dropFirst(seFilter$sd)*1.96,
           dropFirst(LFilter$m[,1]) - dropFirst(seFilter$sd)*1.96,
```

```
            dropFirst(LSmooth$s[,1])), start=c(2009,1)),
     plot.type="s", col=c(1,rep(2,3),3),
     main="Listeria Cases", ylab="",
     xlab="")
# Plot the seasonal component
plot(ts(rowSums(dropFirst(LFilter$m[,2:12])), start=c(2008,1), freq=12),
     xlab="", ylab="", main="Seasonal Component")
abline(h=0)
# Residuals
r <- residuals(LFilter)
plot(dropFirst(r$res), plot.type="s", xlab="Time", ylab="", main="Residuals")
abline(h=0)
# Check that the residuals are white noise - Figure not shown, but used as
diagnosis of the model
tsdiag(LFilter, gof.lag=24)
Box.test(r$res, type="Ljung")
Box.test(r$res, lag=2, type="Ljung")
Box.test(r$res, lag=12, type="Ljung")
# So now that we know we have white noise residuals, use this model to
# generate the forecasts we want.
FL <- dlmForecast(LFilter, nAhead=84)
# Plot the forecasts - Figure in the report
par(mfrow=c(1,1), mai=c(1.2,1.2,0.5,0.5))
plot(L, xlim=c(2007,2022), ylim=c(50,270),  xlab="Time",ylab="Number of
cases/month"
 #    , main="Aggregate Listeria Predictions, +/- 1 sd."
)
lines(FL$f, col=2)
Qv <- unlist(FL$Q)
lines(FL$f+sqrt(Qv), col=2, lty=2)
lines(FL$f-sqrt(Qv), col=2, lty=2)
########################################################
# Local trend model
########################################################
# initial conditions for the filter
level0 <- L[1]
slope0 <- mean(diff(L))
buildm1 <- function(u) {
  # Start with a local linear trend, random walk model
  # We will estimate the state-space variances here as
  # args u[1:2]
  trend <- dlmModPoly(dV = 1e-7, dW = exp(u[1:2]),
                      m0 = c(level0, slope0),
                      C0 = 2 * diag(2))
  # Now add deterministic seasonal dummies and estimate the
  # variation around these as exp(u[3])
  seas <- dlmModSeas(freq=12, dV=exp(u[3]))  # det. seasonal
  return(trend + seas)
}
# Initialize and estimate via MLE
init <- rep(0,3)
outMLE1 <- dlmMLE(L, init, buildm1, hessian=TRUE)
# Look at the MLE output and the standard errors
# Here you get V, U and W
print(exp(outMLE1$par))
sqrt(diag(solve(outMLE1$hessian)))
dlmL1 <- buildm1(outMLE1$par)
# Inspect the parameters
# Variances of the state equation
sqrt(diag(W(dlmL))[c(1:2)])
# Generate the in-sample filtered and smoothed DLMS
L1Filter <- dlmFilter(L, dlmL1)
L1Smooth <- dlmSmooth(L1Filter)
# Plot out the results for the trend model
par(mfrow=c(3,1), mai=c(0.4,0.5,0.5,0.15), cex.axis=1.5)
plot(cbind(L,dropFirst(L1Filter$m[,1])+dropFirst(L1Filter$m[,2]),
           dropFirst(L1Smooth$s[,1]) + dropFirst(L1Smooth$s[,2])),
     plot.type="s", col=1:3, main="Listeria Cases", ylab="",
```

```
     xlab="")
 legend(2008, 220, c("Data", "Filter Trend", "Smoothed Trend"),
       lty=1, col=1:3, cex=0.85)
# Plot the seasonal component
plot(ts(rowSums(dropFirst(L1Filter$m[,3:13])), start=c(2007,1), freq=12),
     xlab="", ylab="", main="Seasonal Component")
abline(h=0)
# Residuals
r <- residuals(L1Filter)
plot(dropFirst(r$res), plot.type="s", xlab="", ylab="", main="Residuals")
abline(h=0)
# Check the residuals
tsdiag(L1Filter, gof.lag=24)
Box.test(r$res, type="Ljung")
Box.test(r$res, lag=2, type="Ljung")
Box.test(r$res, lag=12, type="Ljung")
# Trying a changepoint model: a common thing
# when ARIMA models are non-stationary
library(strucchange)
breakpoints(L ~ 1, breaks=5) # finds one break in 2013(5)
plot(L)
abline(v=2013+(5/12))
# Look at ACFs and PACFs before and after the break
par(mfcol=c(2,2))
acf(window(L, end=c(2013,4)), main="ACF through 2013(4)")
pacf(window(L, end=c(2013,4)), main="PACF through 2013(4)")
acf(window(L, start=c(2013,5)), main="ACF after 2013(4)")
pacf(window(L, start=c(2013,5)), main="PACF after 2013(4)")
# Look like AR(2) processes with a seasonal lag or two
# Set up the lags
dd <- cbind(L, lag(L, k=-1), lag(L, k=-2), lag(L, k=-12))
dd <- window(dd, start=c(2008,1), end=c(2014,1))
colnames(dd) <- c("L", "L1", "L2", "L12")
# Now fit the regression with breaks
mbp <- breakpoints(L ~ L1 + L2 + L12, data=dd, breaks=5)
plot(mbp) # optimal model is no breaks, so this would lead us back to ARIMA
          # which does not work.

################################
################################
# Part 2: Disaggregated analysis
################################
################################
remove(list=ls())
# Read in the data (this dataset was created from the initial dataset)
L <- read.csv("merged_eu_wide.csv", sep=";")
# Check the count: should be 14002
sum(L[,2:15])
# Now clean up and organize as incidence time series
L <- L[,-1] # Remove the date column
# names(L)
Lind <- L[,1:14]/(L[,15:28]/1e6)  # counts / population / million
# Clean up the variable names
c1 <- gsub(".cases", "", colnames(Lind))
c2 <- gsub("X", "", c1)
# Revised column names
colnames(Lind) <- c2
# Make as a time series
Lind <- ts(Lind, start=c(2008,1), freq=12)
# Split by male and female groups
Lind.male <- Lind[,c(2,4,6,8,10,12,14)]
Lind.female <- Lind[,c(1,3,5,7,9,11,13)]
# Plots (not shown in report)
plot(Lind.male, type="s", main="Listeria incidence by age cohort in millions,
Males")
plot(Lind.female, type="s", main="Listeria incidence by age cohort in millions,
Females")
# Check ACFs
```

```
par(mfcol=c(4,2), mai=c(0.3,0.4,0.5,0.1), omi=c(0,0,1,0))
for(i in 1:7) {acf(Lind.male[,i], main=colnames(Lind.male)[i])}
title("ACFs for Male incidence per million, by age", outer=T)
par(mfcol=c(4,2), mai=c(0.3,0.4,0.5,0.1), omi=c(0,0,1,0))
for(i in 1:7) {acf(Lind.female[,i], main=colnames(Lind.female)[i])}
title("ACFs for Female incidence per million, by age", outer=T)
# Nothing here provides any evidence of a trend in the incidences,
# since the ACFs are decay quickly.

# Now look at the counts
# Set these up as time series
Lc <- ts(L[,1:14], start=c(2008,1), freq=12)
Lc.male <- ts(L[,c(2,4,6,8,10,12,14)], start=c(2008,1), freq=12)
Lc.female <- ts(L[,c(1,3,5,7,9,11,13)], start=c(2008,1), freq=12)
# Plot the ones for the males and females
plot(Lc.male, type="s", main="Listeria counts by age cohort, Males")
plot(Lc.female, type="s", main="Listeria counts by age cohort, Females")

# Now check the ACFs on the counts
source("pests.r")
par(mfcol=c(4,2), mai=c(0.3,0.4,0.5,0.1), omi=c(0,0,1,0))
for(i in 1:7) {counts.acf(Lc.male[,i], main=colnames(Lc.male)[i])}
title("ACFs for Male counts", outer=T)
par(mfcol=c(4,2), mai=c(0.3,0.4,0.5,0.1), omi=c(0,0,1,0))
for(i in 1:7) {counts.acf(Lc.female[,i], main=colnames(Lc.female)[i])}
title("ACFs for Female counts", outer=T)

# Only ACF dynamics to be modelled in oldest age cohorts probably.
# Fit PAR() models (adding more lags than 1 maybe not needed).  These models
include
# a trend (not in log) and the log population in millions as controls

# Generate the log population in millions measures
# New Lpop and pop
Lpop <- ts(log((L[,15:28])), start=c(2008,1), freq=12)
pop <- ts(((L[,15:28])), start=c(2008,1), freq=12)
pop.female <- (pop[,c(1,3,5,7,9,11,13)])
pop.male <- (pop[,c(2,4,6,8,10,12,14)])

###################################
###########Females#################
###################################
# calculate the IRR from a naive Poisson model
# [getting an average increase for the models that have a significant PAR trend].
# Females Last colom in a Report Table
out<-NULL
for(i in 1:7)
{
  yes<- (1:nrow(Lc.female))
  modell<-glm(Lc.female[,i] ~ 1 + yes + offset(log(pop.female[,i])),
              family=poisson())
  out<-rbind(out, c(round(exp(coef(modell))[2],4),
round(exp(confint(modell)[2,]),4)))
}
(out-1)*100
Lc.female[1,]
#####################################################################
# PAR() and Glm models: when Par Model not appropriate Glm model used (only final
selection shown)
yes<- (1:nrow(Lc.female))
Female0104 <- glm(Lc.female[,1] ~ 1 + yes +
offset(log(pop.female[,1])),family=poisson())
Female0514 <- glm(Lc.female[,2] ~ 1 + yes +
offset(log(pop.female[,2])),family=poisson())
Female1524  <- Parp(Lc.female[,3] ~ 1 + yes + offset(log(pop.female[,3])),p=1)
Female2544 <- Parp(Lc.female[,4] ~ 1 + yes + offset(log(pop.female[,4])), p=1)
Female4564 <- Parp(Lc.female[,5] ~ 1 + yes + offset(log(pop.female[,5])), p=1)
Female6574 <- Parp(Lc.female[,6] ~ 1 + yes + offset(log(pop.female[,6])), p=1)
```

```
Female75 <- Parp(Lc.female[,7] ~ 1 + yes + offset(log(pop.female[,7])), p=1)
# Print out the results
summary(Female0104)
summary(Female0514)
summary(Female1524)
summary(Female2544)
summary(Female4564)
summary(Female6574)
summary(Female75)
# Compute the plot the different estimated trends for Females
set.seed(123)
Female0104.t <- glm.trends(Female0104, n=1000)
Female0514.t <- glm.trends(Female0514, n=1000)
Female1524.t <- parp.trends(Female1524, trend=1:nrow(Lc.female), n=1000)
Female2544.t <- parp.trends(Female2544, trend=1:nrow(Lc.female), n=1000)
Female4564.t <- parp.trends(Female4564, trend=1:nrow(Lc.female), n=1000)
Female6574.t <- parp.trends(Female6574, trend=1:nrow(Lc.female), n=1000)
Female75.t <- parp.trends(Female75, trend=log(1:nrow(Lc.female)), n=1000)
# Summarize for plotting:
# Set the CI width
probs <- c(0.025, 0.5, 0.975)
Female.trends <- cbind(t(apply(Female0104.t, 1, quantile, probs=probs)),
                       t(apply(Female0514.t, 1, quantile, probs=probs)),
                       t(apply(Female1524.t, 1, quantile, probs=probs)),
                       t(apply(Female2544.t, 1, quantile, probs=probs)),
                       t(apply(Female4564.t, 1, quantile, probs=probs)),
                       t(apply(Female6574.t, 1, quantile, probs=probs)),
                       t(apply(Female75.t, 1, quantile, probs=probs)))
Female.trends <- ts(Female.trends, start=c(2008,1), freq=12)
# Plot
par(mfrow=c(1,1))
plot(Female.trends, plot.type="s", col=rep(1:7, each=3), lty=c(2,1,2), lwd=2,
     ylab="", main="Female trends")
legend(2008, 40, c("01-04","05-14", "15-24", "25-44", "45-64", "65-74", "75"),
       col=1:7, lty=1, cex=0.8, bty="n")
####################################
###########Males#########################
####################################
# calculate the IRR from the Poisson model.
out<-NULL
for(i in 1:7)
{
  yes<- (1:nrow(Lc.male))
  modell<-glm(Lc.male[,i] ~ 1 + yes + offset(log(pop.male[,i])),
              family=poisson())
  out<-rbind(out, c(round(exp(coef(modell))[2],4),
round(exp(confint(modell)[2,]),4)))
}
out
(out-1)*100
# Male
yes<-(1:nrow(Lc.male))
Male0104<- glm(Lc.male[,1] ~ 1 + yes+ offset(log(pop.male[,1])), family=poisson())
Male0514<- glm(Lc.male[,2] ~ 1 + yes+ offset(log(pop.male[,2])), family=poisson())
Male1524 <- glm(Lc.male[,3] ~ 1 + yes + offset(log(pop.male[,3])),
                family=poisson())
Male2544<- Parp(Lc.male[,4] ~ 1 + yes + offset(log(pop.male[,4])), p=2)
Male2544g <- glm(Lc.male[,4] ~ 1 + yes + offset(log(pop.male[,4])),
                family=poisson())
Male4564 <- Parp(Lc.male[,5] ~ 1 + yes + offset(log(pop.male[,5])), p=1)
Male6574 <- Parp(Lc.male[,6] ~ 1 + yes + offset(log(pop.male[,6])),  p=2)
Male75<- Parp(Lc.male[,7] ~ 1 + yes + offset(log(pop.male[,7])),  p=2)
# Print out the results
summary(Male0104)
summary(Male0514)
summary(Male1524)
summary(Male2544)
summary(Male2544g)
```

```
summary(Male4564)
summary(Male6574)
summary(Male75)
# Compute the plot the different estimated trends for Males
set.seed(123)
Male0104.t <- glm.trends(Male0104, n=1000)
Male0514.t <- glm.trends(Male0514, n=1000)
Male1524.t <- glm.trends(Male1524, n=1000)
Male2544.t <- parp.trends(Male2544, trend=(1:nrow(Lc.male)), n=1000)
Male2544g.t <- glm.trends(Male2544g, n=1000)
Male4564.t <- parp.trends(Male4564, trend=(1:nrow(Lc.male)), n=1000)
Male6574.t <- parp.trends(Male6574, trend=(1:nrow(Lc.male)), n=1000)
Male75.t <- parp.trends(Male75, trend=(1:nrow(Lc.male)), n=1000)
# Now summarize for plotting:
# Set the CI width --> Check Male2544.t
probs <- c(0.025, 0.5, 0.975)
Male.trends <- cbind(t(apply(Male0104.t, 1, quantile, probs=probs)),
                     t(apply(Male0514.t, 1, quantile, probs=probs)),
                     t(apply(Male1524.t, 1, quantile, probs=probs)),
                   t(apply(Male2544g.t, 1, quantile, probs=probs)),
                     t(apply(Male4564.t, 1, quantile, probs=probs)),
                     t(apply(Male6574.t, 1, quantile, probs=probs)),
                     t(apply(Male75.t, 1, quantile, probs=probs)))
Male.trends <- ts(Male.trends, start=c(2008,1), freq=12)
# Now plot:
plot(Male.trends,
     plot.type="s", col=rep(1:7, each=3), lty=c(2,1,2), lwd=2,
     ylab="", main="Male trends")
legend(2008, 40, c("01-04","05-14", "15-24", "25-44", "45-64", "65-74", "75"),
       col=1:6, lty=1, cex=0.8, bty="n")

#########################################################################
# Now plot the fitted models against the data so we can see the changes in
# the mean and trend. This is a lot of code to make a single custom plot!
#########################################################################
par(mfcol=c(4,2), mai=c(0.3,0.4,0.5,0.1), omi=c(0,0,1,0))
# Start with Females by age group
# Female0104
Female0104fit <- predict(Female0104, type = "response", se=TRUE)
plot.ts(cbind(Lc.female[,1],
              ts(cbind(Female0104fit$fit,
                       Female0104fit$fit + Female0104fit$se,
                       Female0104fit$fit - Female0104fit$se),
                 start=c(2008,1), freq=12)),
        plot.type="s", col=c(1,2,2,2), lty=c(1,1,2,2),
        main="Female, 01-04", ylab="")
abline(h=mean(Lc.female[,1]))

# Female0514
Female0514fit <- predict(Female0514, type = "response", se=TRUE)
plot.ts(cbind(Lc.female[,2],
              ts(cbind(Female0514fit$fit,
                       Female0514fit$fit + Female0514fit$se,
                       Female0514fit$fit - Female0514fit$se),
                 start=c(2008,1), freq=12)),
        plot.type="s", col=c(1,2,2,2), lty=c(1,1,2,2),
        main="Female, 05-14", ylab="")
abline(h=mean(Lc.female[,2]))

# Female1524
plot.ts(cbind(Lc.female[,3],
              ts(cbind(Female1524$fit[,1],
                       Female1524$fit[,1] + sqrt(Female1524$fit[,2]),
                       Female1524$fit[,1] - sqrt(Female1524$fit[,2])),
                 start=c(2008,1), freq=12)),
        plot.type="s", col=c(1,2,2,2), lty=c(1,1,2,2),
        main="Female, 15-24", ylab="")
abline(h=mean(Lc.female[,3]))
```

```
# Female2544
plot.ts(cbind(Lc.female[,4],
              ts(cbind(Female2544$fit[,1],
                       Female2544$fit[,1] + sqrt(Female2544$fit[,2]),
                       Female2544$fit[,1] - sqrt(Female2544$fit[,2])),
                 start=c(2008,1), freq=12)),
        plot.type="s", col=c(1,2,2,2), lty=c(1,1,2,2),
        main="Female, 25-44", ylab="")
abline(h=mean(Lc.female[,4]))

# Female4564
plot.ts(cbind(Lc.female[,5],
              ts(cbind(Female4564$fit[,1],
                       Female4564$fit[,1] + sqrt(Female4564$fit[,2]),
                       Female4564$fit[,1] - sqrt(Female4564$fit[,2])),
                 start=c(2008,1), freq=12)),
        plot.type="s", col=c(1,2,2,2), lty=c(1,1,2,2),
        main="Female, 45-64", ylab="")
abline(h=mean(Lc.female[,5]))

# Female6574
plot.ts(cbind(Lc.female[,6],
              ts(cbind(Female6574$fit[,1],
                       Female6574$fit[,1] + sqrt(Female6574$fit[,2]),
                       Female6574$fit[,1] - sqrt(Female6574$fit[,2])),
                 start=c(2008,1), freq=12)),
        plot.type="s", col=c(1,2,2,2), lty=c(1,1,2,2),
        main="Female, 65-74", ylab="")
abline(h=mean(Lc.female[,6]))

# Female75+
plot.ts(cbind(Lc.female[,7],
              ts(cbind(Female75$fit[,1],
                       Female75$fit[,1] + sqrt(Female75$fit[,2]),
                       Female75$fit[,1] - sqrt(Female75$fit[,2])),
                 start=c(2008,1), freq=12)),
        plot.type="s", col=c(1,2,2,2), lty=c(1,1,2,2),
        main="Female, 75+", ylab="")
abline(h=mean(Lc.female[,7]))

title(main="Female Cohorts, 2008-2015", outer=TRUE)

# Same kind of plot for the Males...
par(mfcol=c(4,2), mai=c(0.3,0.4,0.5,0.1), omi=c(0,0,1,0))

# Male0104
Male0104fit <- predict(Male0104, type = "response", se=TRUE)
plot.ts(cbind(Lc.male[,1],
              ts(cbind(Male0104fit$fit,
                       Male0104fit$fit + Male0104fit$se,
                       Male0104fit$fit - Male0104fit$se),
                 start=c(2008,1), freq=12)),
        plot.type="s", col=c(1,2,2,2), lty=c(1,1,2,2),
        main="Male, 01-04", ylab="")
abline(h=mean(Lc.male[,1]))

# Male0514
Male0514fit <- predict(Male0514, type = "response", se=TRUE)
plot.ts(cbind(Lc.male[,2],
              ts(cbind(Male0514fit$fit,
                       Male0514fit$fit + Male0514fit$se,
                       Male0514fit$fit - Male0514fit$se),
                 start=c(2008,1), freq=12)),
        plot.type="s", col=c(1,2,2,2), lty=c(1,1,2,2),
        main="Male, 05-14", ylab="")
abline(h=mean(Lc.male[,2]))
```

```
# Male1524
Male1524fit <- predict(Male1524, type = "response", se=TRUE)
plot.ts(cbind(Lc.male[,3],
              ts(cbind(Male1524fit$fit,
                       Male1524fit$fit + Male1524fit$se,
                       Male1524fit$fit - Male1524fit$se),
                 start=c(2008,1), freq=12)),
        plot.type="s", col=c(1,2,2,2), lty=c(1,1,2,2),
        main="Male, 15-24", ylab="")
abline(h=mean(Lc.male[,3]))

par(mfrow=c(2,1))
# Male2544 -- PAR - model
plot.ts(cbind(Lc.male[,4],
              ts(cbind(Male2544$fit[,1],
                       Male2544$fit[,1] + sqrt(Male2544$fit[,2]),
                       Male2544$fit[,1] - sqrt(Male2544$fit[,2])),
                 start=c(2008,1), freq=12)),
        plot.type="s", col=c(1,2,2,2), lty=c(1,1,2,2),
        main="Male, 25-44 - par", ylab="")
abline(h=mean(Lc.male[,4]))

# Male2544 -- GLM - model
Male2544gfit <- predict(Male2544g, type = "response", se=TRUE)
plot.ts(cbind(Lc.male[,4],
              ts(cbind(Male2544gfit$fit,
                       Male2544gfit$fit + Male2544gfit$se,
                       Male2544gfit$fit - Male2544gfit$se),
                 start=c(2008,1), freq=12)),
        plot.type="s", col=c(1,2,2,2), lty=c(1,1,2,2),
        main="Male, 25-44 -glm", ylab="")
abline(h=mean(Lc.male[,4]))

# Male4564
plot.ts(cbind(Lc.male[,5],
              ts(cbind(Male4564$fit[,1],
                       Male4564$fit[,1] + sqrt(Male4564$fit[,2]),
                       Male4564$fit[,1] - sqrt(Male4564$fit[,2])),
                 start=c(2008,1), freq=12)),
        plot.type="s", col=c(1,2,2,2), lty=c(1,1,2,2),
        main="Male, 45-64", ylab="")
abline(h=mean(Lc.male[,5]))

# Male6574
plot.ts(cbind(Lc.male[,6],
              ts(cbind(Male6574$fit[,1],
                       Male6574$fit[,1] + sqrt(Male6574$fit[,2]),
                       Male6574$fit[,1] - sqrt(Male6574$fit[,2])),
                 start=c(2008,1), freq=12)),
        plot.type="s", col=c(1,2,2,2), lty=c(1,1,2,2),
        main="Male, 65-74", ylab="")
abline(h=mean(Lc.male[,6]))

# Male75+
plot.ts(cbind(Lc.male[,7],
              ts(cbind(Male75$fit[,1],
                       Male75$fit[,1] + sqrt(Male75$fit[,2]),
                       Male75$fit[,1] - sqrt(Male75$fit[,2])),
                 start=c(2008,1), freq=12)),
        plot.type="s", col=c(1,2,2,2), lty=c(1,1,2,2),
        main="Male, 75+", ylab="")
abline(h=mean(Lc.male[,7]))

title(main="Male Cohorts, 2008-2015", outer=TRUE)

# Now check all of the residuals for serial correlation (there should be -nearly-
none)
par(mfcol=c(4,2), mai=c(0.3,0.4,0.5,0.1), omi=c(0,0,1,0))
```

```
acf(Female0104$residuals)
acf(residuals(Female0514))
acf(residuals(Female1524))
acf(Female2544$residuals)
acf(Female4564$residuals)
acf(Female6574$residuals)
acf(Female75$residuals)
title(main="Female Cohorts, residuals ACFs", outer=TRUE)

par(mfcol=c(4,2), mai=c(0.3,0.4,0.5,0.1), omi=c(0,0,1,0))
acf(Male0104$residuals)
acf(residuals(Male0514))
acf(residuals(Male1524))
acf(Male2544$residuals)
acf(Male4564$residuals)
acf(Male6574$residuals)
acf(Male75$residuals)
title(main="Male Cohorts, residuals ACFs", outer=TRUE)

#########################################################################
## EU PLOT Smoothed >>>>>> Report Figure
#########################################################################
remove(list=ls())
cases_eu <- read.csv2("merged_eu.csv")
cases_eu$rate <- cases_eu$NumberOfCases / cases_eu$pop
cases_eu$Time <-
  as.Date(paste("01", cases_eu$Month, cases_eu$Year), "%d %m %Y")
sum(cases_eu$NumberOfCases)
#############
## packages
#############
library(ggplot2)
library(RColorBrewer)
levels(cases_eu$AgeGroup_ECDC) <- gsub("X", "", levels(cases_eu$AgeGroup_ECDC))
ggplot(cases_eu, aes(x = Time, y = rate)) +
  geom_line(aes(col = Gender)) +
  facet_wrap(~AgeGroup_ECDC, ncol = 2, scales = "free") +
  geom_smooth(se = FALSE, aes(col = Gender)) +
  ylab("Listeriosis incidence rates")+
  xlab("Time")+
  ggtitle("")+
  theme_bw() +
  coord_cartesian(ylim = c(0, 3e-6))
ggplot(cases_eu, aes(x = Time, y = rate)) +
  geom_line(aes(col = Gender)) +
  facet_wrap(~AgeGroup_ECDC, ncol = 2, scales = "free") +
  geom_smooth(se = FALSE, aes(col = Gender)) +
  ylab("Listeriosis incidence rates")+
  xlab("Time")+
  ggtitle("")+
  theme_bw()
## EU PLOT2 >>>2 Age Groups >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
cases_eu2 <- subset(cases_eu, AgeGroup_ECDC == "75+" | AgeGroup_ECDC == "65-74")
ggplot(cases_eu2, aes(x = Time, y = rate)) +
  geom_line(aes(col = Gender)) +
  facet_wrap(~AgeGroup_ECDC, ncol = 1, scales = "free") +
  geom_smooth(se = FALSE, aes(col = Gender)) +
# ggtitle("Listeria incidence rates - Europe") +
  theme_bw() +
  coord_cartesian(ylim = c(0, 2.8e-6))
 # Comparison of groups
#############
## packages
#############
library(epitools)
# Check once again the numbers, should be 14002
sum(cases_eu$NumberOfCases)
cases_eu$Gender <- factor(cases_eu$Gender)
```

```
###
### EUROPE
###
all_age <- unique(cases_eu$AgeGroup_ECDC)
out <-
  data.frame(age = NULL,
             male = NULL,
             female = NULL,
             ratio = NULL,
             p.value = NULL)

for (age in seq_along(all_age)) {
  count <-
    with(subset(cases_eu, Year == 2008 &
                  AgeGroup_ECDC == all_age[age]),
         c(tapply(NumberOfCases, Gender, sum)))
  pop <-
    with(subset(cases_eu, Year == 2008 &
                  AgeGroup_ECDC == all_age[age]),
         c(tapply(pop, Gender, sum)))
  rate <- count/pop
  if (any(count == 0)) {
    p <- r <- rl <- ru <- NA

  } else {
    res <- rateratio(x = count, y = pop)
    p <- res$p.value[2, 1]
    r <- res$measure[2, 1]
    rl <- res$measure[2, 2]
    ru <- res$measure[2, 3]
  }
    out <- rbind(out,
                 data.frame(age = all_age[age],
                            male = round(rate[2]*1000000,2),
                            female = round(rate[1]*1000000,2),
                            ratio = round(r,2),
                            ratio.lwr = round(rl,2),
                            ratio.upr = round(ru,2),
                            p.value = round(p,3)))
}
out
```

The evolution of reported human listeriosis incidence rates in the EU/EEA (period 2008–2015) is shown in Figure 32.

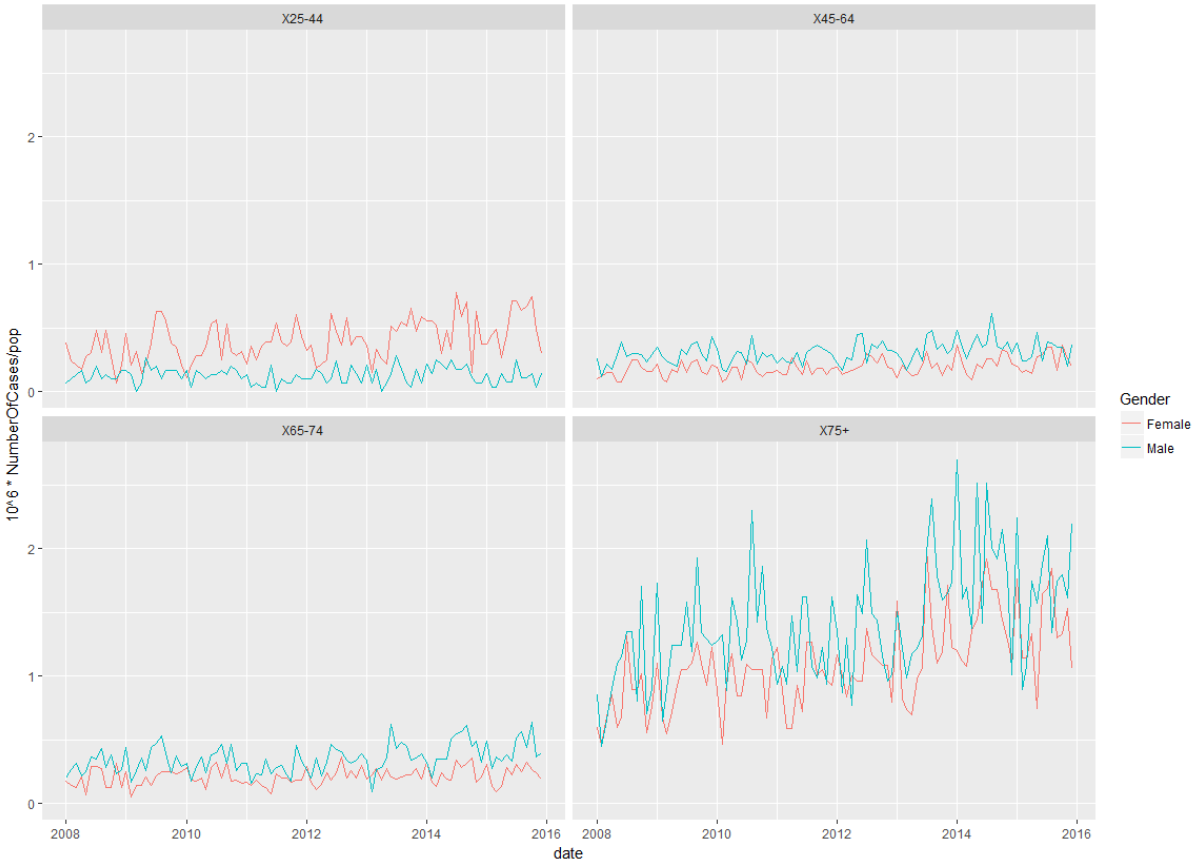


Figure 32: Evolution of reported human listeriosis incidence rates (cases per month/1,000,000 population) in the EU/EEA, by gender for a selection of age groups, 2008–2015

Appendix C – Additional information of the *Listeria monocytogenes* generic QMRA (gQMRA) model

The R-code of the *Listeria monocytogenes* generic quantitative microbiological risk assessment (gQMRA) model is included below as well as the inputs of the gQMRA model (Tables 30-37). The latter are included in an Excel file containing tables.

Table 30: Data used for calculating the prevalence of *L. monocytogenes* contamination of the 13 ready-to-eat (RTE) subcategories/packaging conditions ('prev' table)

RTE category	RTE subcategory	Packaging	group[(a)]	N	S	groupc[(b)]
Fish products	Cold smoked fish	ROP	1	613	94	Smoked fish
Fish products	Hot smoked fish	ROP	2	512	32	Smoked fish
Fish products	Gravad fish	ROP	3	252	30	Gravad fish
Meat products	Cooked meat	ROP	4	2490	46	Cooked meat
Meat products	Sausage	ROP	5	762	13	Sausage
Meat products	Pâté	ROP	6	184	9	Pâté
Fish products	Cold smoked fish	normal	7	613	94	Smoked fish
Fish products	Hot smoked fish	normal	8	512	32	Smoked fish
Fish products	Gravad fish	normal	9	252	30	Gravad fish
Meat products	Cooked meat	normal	10	2490	46	Cooked meat
Meat products	Sausage	normal	11	762	13	Sausage
Meat products	Pâté	normal	12	184	9	Pâté
Cheese	Soft and semi-soft cheese	normal	13	3114	13	Soft and semi-soft cheese

N: total number of samples; ROP: reduced oxygen packaging; S: number of positive samples.
(a): This is the designation to the Group used for further calculations.
(b): This is the designation to the Groupc used for further calculations.

Table 31: Total number of eating occasions per year for the seven ready-to-eat (RTE) subcategories for the 14 subpopulation groups in the EU/EEA ('conso' table)

Age	Gender	Smoked fish	Gravad fish	Cooked meat	Sausage	Pâté	Soft and semi-soft cheese	Pop u-latio n[(a)]
01–04	Female	2.71E+08	6.03E+07	7.49E+08	1.00E+09	5.91E+08	2.32E+08	1
01–04	Male	3.06E+08	6.83E+07	8.64E+08	9.82E+08	6.50E+08	2.02E+08	2
05–14	Female	2.22E+08	4.95E+07	2.49E+09	2.44E+09	9.58E+08	4.75E+08	3
05–14	Male	2.25E+08	5.03E+07	2.78E+09	2.84E+09	1.21E+09	4.75E+08	4
15–24	Female	3.98E+08	8.87E+07	2.79E+09	1.64E+09	6.71E+08	6.79E+08	5
15–24	Male	2.63E+08	5.87E+07	4.05E+09	2.71E+09	1.06E+09	5.93E+08	6
25–44	Female	8.31E+08	1.85E+08	8.45E+09	4.70E+09	1.64E+09	2.30E+09	7
25–44	Male	9.33E+08	2.08E+08	1.13E+10	7.66E+09	2.89E+09	2.03E+09	8
45–64	Female	1.39E+09	3.10E+08	9.21E+09	5.29E+09	1.59E+09	2.46E+09	9
45–64	Male	1.57E+09	3.49E+08	1.16E+10	8.03E+09	2.73E+09	2.56E+09	10
65–74	Female	1.01E+09	2.24E+08	3.87E+09	2.05E+09	7.82E+08	1.05E+09	11
65–74	Male	9.94E+08	2.22E+08	4.00E+09	2.40E+09	1.08E+09	1.05E+09	12
75+	Female	1.59E+09	3.54E+08	3.56E+09	2.02E+09	1.23E+09	1.33E+09	13
75+	Male	1.57E+09	3.51E+08	2.78E+09	1.99E+09	1.18E+09	1.18E+09	14

(a): This is the designation to the Population used for further calculations.

Table 32: Portion size (mass of RTE food ingested per meal; in grams) for the seven ready-to-eat (RTE) subcategories for the 14 subpopulation groups in the EU/EEA ('size' table)

Age	Gender	Smoked fish	Gravad fish	Cooked meat	Sausage	Pâté	Soft and semi-soft cheese
01–04	Female	26	26	22	38	19	21
01–04	Male	21	21	23	44	22	20
05–14	Female	54	54	31	54	28	27
05–14	Male	56	56	32	63	29	43
15–24	Female	56	56	39	68	36	40
15–24	Male	57	57	51	90	49	43
25–44	Female	64	64	42	61	41	48
25–44	Male	78	78	53	79	53	45
45–64	Female	61	61	42	63	41	46
45–64	Male	87	87	53	78	49	44
65–74	Female	60	60	40	55	31	32
65–74	Male	58	58	42	70	44	40
75+	Female	49	49	30	63	33	36
75+	Male	66	66	42	61	38	41

Table 33: *L. monocytogenes* concentrations (in $\log_{10}$ CFU/g) per RTE food subcategory ('conc' table)

RTE category	RTE subcategory	Packaging	group	min	Max	shape1	shape2
Fish products	Smoked fish	ROP	1	-1.69	5	0.684	2.655
Fish products	Hot smoked fish	ROP	2	-1.69	6	0.684	2.655
Fish products	Gravad fish	ROP	3	-1.69	6	1.210	5.450
Meat products	Cooked meat	ROP	4	-1.69	6	0.502	2.908
Meat products	Sausage	ROP	5	-1.69	6	0.502	2.908
Meat products	Pâté	ROP	6	-1.69	6	0.502	2.908
Fish products	Cold smoked fish	normal	7	-1.69	5	0.684	2.655
Fish products	Hot smoked fish	normal	8	-1.69	6	0.684	2.655
Fish products	Gravad fish	normal	9	-1.69	6	1.210	5.450
Meat products	Cooked meat	normal	10	-1.69	6	0.502	2.908
Meat products	Sausage	normal	11	-1.69	6	0.502	2.908
Meat products	Pâté	normal	12	-1.69	6	0.502	2.908
Cheese	Soft and semi-soft cheese	normal	13	-1.69	7	0.194	3.177

ROP: reduced oxygen packaging. *Listeria monocytogenes* concentrations (at decimal logarithm scale) in RTE food were modelled using beta-general distributions with a minimum equal to -1.69 and maximum equal to 6.1. The two other (shape) parameters of the food-specific beta-general distributions (α and β) were estimated using a maximum likelihood estimation algorithm implemented in the 'mle' function ('stats4' package in R version 3.3.3 (Ihaka and Gentleman, 1996; R Core Team, 2016)).

Table 34: The exponential growth rate (EGR) at 5°C for the 13 ready-to-eat (RTE) subcategories/packaging conditions ('EGR' table)

RTE category	RTE subcategory	Packaging	group	min	Max	m	sd	shift	Nmax. mean	Nmax .min	Nmax. max
Fish products	Cold smoked fish	ROP	1	0	0.0686	0.017081	0.013619	-0.0030763	7.29	7.00	8.98
Fish products	Hot smoked fish	ROP	2	0	0.0686	0.017081	0.013619	-0.0030763	7.29	7.00	8.98
Fish products	Gravad fish	ROP	3	0	0.0686	0.017081	0.013619	-0.0030763	7.29	7.00	8.98
Meat products	Cooked meat	ROP	4	0	0.087206	0.021793	0.017664	-0.0013931	6.23	3.37	8.91
Meat products	Sausage	ROP	5	0	0.087206	0.021793	0.017664	-0.0013931	6.23	3.37	8.91
Meat products	Pâté	ROP	6	0	0.023	0.014	0.005	0	7.53	4.02	9.00
Fish products	Cold smoked fish	normal	7	0	0.0617	0.011959	0.01073	-0.0004839	7.29	7.00	8.98
Fish	Hot smoked	normal	8	0	0.0617	0.011959	0.01073	-0.0004839	7.29	7.00	8.98

products	fish										
Fish products	Gravad fish	normal	9	0	0.0617	0.011959	0.01073	-0.0004839	7.29	7.00	8.98
Meat products	Cooked meat	normal	10	0	0.086484	0.025698	0.019291	-0.0026281	6.23	3.37	8.91
Meat products	Sausage	normal	11	0	0.086484	0.025698	0.019291	-0.0026281	6.23	3.37	8.91
Meat products	Pâté	normal	12	0	0.097017	0.025697	0.0098129	0.005627	7.53	4.02	9.00
Cheese	Soft and semi-soft cheese	normal	13	0	0.0296338 48	0.010293	0.01508	0.0008631 1	7.28	7.00	8.99

ROP: reduced oxygen packaging. It was assumed that the exponential growth rate (EGR) at 5°C is log-normally distributed.

Table 35: Remaining shelf life (in days) for the 13 ready-to-eat (RTE) subcategories/packaging conditions ('r_time' table)

RTE category	RTE subcategory	Packaging	group	min	Max	m
Fish products	Cold smoked fish	ROP	1	1	519	23.94
Fish products	Hot smoked fish	ROP	2	2	114	15.25
Fish products	Gravad fish	ROP	3	1	393	21.97
Meat products	Cooked meat	ROP	4	0	427	19.69
Meat products	Sausage	ROP	5	0	143	19.06
Meat products	Pâté	ROP	6	1	99	21.79
Fish products	Cold smoked fish	normal	7	6	37	11.69
Fish products	Hot smoked fish	normal	8	3	42	8.89
Fish products	Gravad fish	normal	9	3	370	86.96
Meat products	Cooked meat	normal	10	1	160	19.13
Meat products	Sausage	normal	11	0	106	15.29
Meat products	Pâté	normal	12	3	149	19.68
Cheese	Soft and semi-soft cheese	normal	13	0	411	33.14

ROP: reduced oxygen packaging.

Table 36: Proportion of reduced oxygen packaging (ROP) and normal packaging for the seven ready-to-eat (RTE) subcategories ('ROP' table)

RTE category	RTE subcategory	RTE2	Packaging	group	P[(a)]	p2[(b)]
Fish products	Cold smoked fish	Smoked fish	ROP	1	0.96	0.7
Fish products	Hot smoked fish	Smoked fish	ROP	2	0.73	0.3
Fish products	Gravad fish	Gravad fish	ROP	3	0.78	1
Meat products	Cooked meat	Cooked meat	ROP	4	0.87	1
Meat products	Sausage	Sausage	ROP	5	0.78	1
Meat products	Pâté	Pâté	ROP	6	0.75	1
Fish products	Cold smoked fish	Cold smoked fish	normal	7	0.04	0.7
Fish products	Hot smoked fish	Hot smoked fish	normal	8	0.27	0.3
Fish products	Gravad fish	Gravad fish	normal	9	0.22	1
Meat products	Cooked meat	Cooked meat	normal	10	0.13	1
Meat products	Sausage	Sausage	normal	11	0.22	1
Meat products	Pâté	Pâté	normal	12	0.25	1
Cheese	Soft and semi-soft cheese	Soft and semi-soft cheese	normal	13	1	1

ROP: reduced oxygen packaging.
(a): This is the fraction for each RTE food subcategory split by packaging type.
(b): This is the fraction for hot and cold-smoked fish.

Table 37: Dose–response model for the 14 subpopulation groups ('DR' table)

Age	Gender	RR	Path	RefSdLog	RefSdLogI	Mean	population[(a)]
01–04	Female	0.17	Female 1–4 yo	1.62	0.55	-14.574	1
01–04	Male	0.20	Male 1–4 yo	1.62	0.55	-14.467	2
05–14	Female	0.07	Female 5–14 yo	1.62	0.55	-14.916	3
05–14	Male	0.07	Male 5–14 yo	1.62	0.55	-15.005	4
15–24	Female	0.26	Female 15–24 yo	1.62	0.55	-14.325	5
15–24	Male	0.08	Male 15–24 yo	1.62	0.55	-15.036	6
25–44	Female	0.54	Female 25–44 yo	1.62	0.55	-14.025	7
25–44	Male	0.18	Male 25–44 yo	1.62	0.55	-14.764	8
45–64	Female	0.63	Female 45–64 yo	1.62	0.55	-14.081	9
45–64	Male	1.07	Male 45–64 yo	1.62	0.55	-14.045	10
65–74	Female	1.87	Female 65–74 yo	1.62	0.55	-13.702	11
65–74	Male	3.50	Male 65–74 yo	1.62	0.55	-13.560	12
75+	Female	3.40	Female >75 yo	1.62	0.55	-13.536	13
75+	Male	6.33	Male >75 yo	1.62	0.55	-13.536	14

RR: relative risk.
(a): This is the designation to the Population used for further calculations.

```
setwd("~/Studio/QMRALMEFSA")
#Option 1 BLS concentration data <- input2.xlsx
#Option 2 US concentration data <- input.xlsx
#Option 3 mix BLS and US data <- input3.xlsx
xfile="inputs3.xlsx"
step=0.1
DoseCont <- seq(0, 12, step)
source("script1.R") #Stochastic dose response model to be run one time
source("script2.R") #full script from initial conc to number of cases

options(digits=4)
#contamfun is defined in script2
rescases=contamfun(runs=1000000,shift=0,meanTemp=5.9,
                   sdTemp=2.9,
                   Mode_prop_rtime=0.3,
                   Max_prop_rtime=1.1)
View(rescases)
sum(rescases$cases)
```

Script1.R

```
#####################################
#defined functions
#####################################

#Stochastic dose response model
DRLNDose <- function(r, Dose, meanlog, sdlog) {
  dnorm(r, meanlog, sdlog) * ((r>=0) *1 + (r<0) * (-expm1(-10^Dose * 10^r)))
}
DR <- function(Dose,meanlog,sdlog,low=-Inf,up=Inf,Print=FALSE,tol=1E-20,...){
  #This function provide the marginal prob of invasive listeriosis
  #in a given population for a Dose Dose in log 10 cfu.
  #the default use the parameters estimated just before
  #and stored in Data
  #note: this function is not vectorized
  res <- "try-error"
  while(res=="try-error"){
    Int <- try(integrate(DRLNDose,
                         lower=low, upper=up,
                         rel.tol=tol,
                         Dose=Dose, meanlog=meanlog, sdlog=sdlog, ...),silent=TRUE)
    res <- class(Int)
    tol <- tol * 10
  }
  if(Print) print(Int)
```

```
  return(Int$value)
}
#######################################
#Conditional dose response
#per age and gender(14 subpopulations)
#######################################

DRP=read_excel(xfile,sheet="DR")
#step=0.1
#DoseCont <- seq(0, 12, step)
cond_risk_fun=function(i){
  sapply(DoseCont,DR,meanlog=DRP$Mean[i],sdlog=DRP$RefSdLog[i])
}
cond_risk=sapply(1:length(unique(DRP$population)),cond_risk_fun)

df_DR=data.frame(cond_risk)%>%
  gather("population",prob,1:ncol(cond_risk))

path=data_frame(population=unique(df_DR$population),Path=DRP$Path)
df_DR=left_join(df_DR,path,key=population)
df_DR$DoseCont=rep(DoseCont,14)
df_DR$Gender=rep(c("Female","Male"),each=nrow(df_DR)/2)
library(readr)

path="save_df_DR"
write_rds(df_DR, path)
```

Script2.R

```
library(readr)
library(readxl)
library(msm)
library(tidyverse)
library(plotly)
library(mc2d)
library(knitr)
library(ggplot2)
library(readr)

#step=0.1
#DoseCont <- seq(0, 12, step)

################################################
#Load the input file
################################################

path="save_df_DR"
df_DR=read_rds(path)

################################################
#Extract inputs from xfile
################################################

prev=read_excel(xfile,sheet="prev")
conc=read_excel(xfile,sheet="conc")
EGR=read_excel(xfile,sheet="EGR")
ROP=read_excel(xfile,sheet="ROP")
DRP=read_excel(xfile,sheet="DR")
conso=read_excel(xfile,sheet="conso")
size=read_excel(xfile,sheet="size")
r_time=read_excel(xfile,sheet="r_time")
################################################
################################################
#defined needed fuctions
################################################

#Convert mean and varaiance of Y to
#mean and std of log(Y)
convert_m_v=function(m,v){
```

```
  phi = sqrt(v + m^2)
  mu=log(m^2/phi) #mean of log(Y)
  sigma = sqrt(log(phi^2/m^2))#
  c(mu,sigma)
}
#Primary growth model
rosso=function(time,egrm,lag=0,x0,xmax){
  x0=10^x0
  xmax=10^xmax
  den=1+(xmax/x0 -1)*exp(-egrm*(time-lag))
  log10(xmax/den)
}
################################################
################################################
####Start of the definition of contamfunc
####shift is argument that shifts the xmax (maximum population density)
### meanTemp and sdTemp are the parameters of the normal distribution of
###the consumer refrigerators
###Mode_prop_rtime and Max_prop_rtime are respectively the mode and the maximum of
the proportion
###of the shelflife used as time of storage
###runs is the number of iterations
contamfun=function(runs,shift=0,meanTemp=5.9,
                   sdTemp=2.9,
                   Mode_prop_rtime=0.3,
                   Max_prop_rtime=1.1) {
  ######################################
  #Initial concentration for each
  #of the 13 RTE subcategories
  ######################################
  C0fun=function(i) rbetagen(runs,
                             shape1=conc$shape1[i],
                             shape2=conc$shape2[i],
                             min=conc$min[i],
                             max=conc$max[i]+shift)
  #the function C0fun is applied to each of the thirteen
  #RTE food categories thanks to sapply
  C0r=sapply(1:nrow(conc),C0fun)
  #C0r is a matrix with a number of lines equal to runs
  #and a number of columns eaqual to the number of RTE food category
  #dimension is runs x 13
  ######################################
  #Exponential Growth Rate
  #for each of the 13 RTE subcategories
  ######################################

  EGR5fun=function(i){
    m=EGR$m[i]
    s=EGR$sd[i]
    parm=convert_m_v(m,s^2)
    rtnorm(runs,mean=parm[1],sd=parm[2],
           lower=log(EGR$min[i]),upper=log(EGR$max[i]))
  }

  EGR5r=exp(sapply(1:nrow(conc),EGR5fun))
  #dimension of EGR5r is runs x 13
  ######################################
  #Refrigerator Temperature
  #EGR
  ######################################

  Tempr=rtnorm(runs,mean=meanTemp,sd=sdTemp,
               lower=-2,upper=15)
  #dimension of Tempr is runs x 1
  Tmin=-1.18
  EGRr=EGR5r*((Tempr-Tmin)/(5-Tmin))^2

  #dimension of EGRr is runs x 13
```

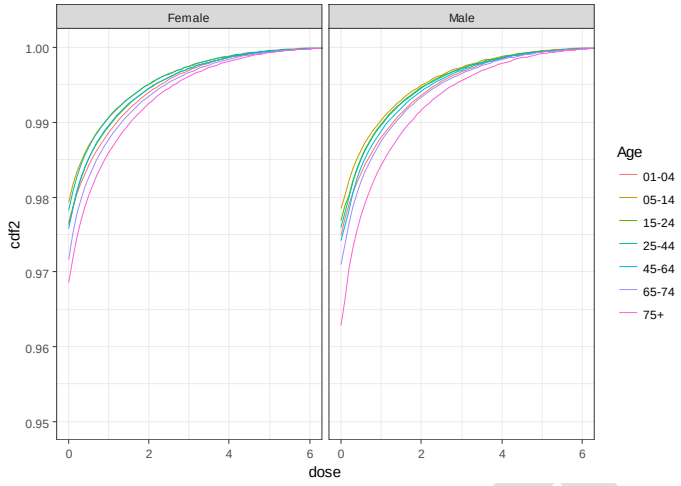
```
#####################################
#Time of storage
#
#####################################

##########
#remaining shelf life
##########
r_timefun=function(i){
  m=r_time$m[i]
  rexp(runs,rate=1/m)
}
r_timer=sapply(1:length(unique(r_time$group)),r_timefun)
#dimension of r_timer is runs x 13
#########
#Proportion of r_time
#########

propr=rpert(runs, min=0, mode=Mode_prop_rtime, max=Max_prop_rtime)
#dimension of propr is runs x 1
#########
#s_time
#########
s_time=r_timer*propr
#dimension of s_time runs x 13
#####################################
#Concnetration at time of consumption
#f_conc
#####################################
f_concfun=function(i){
  Nmax=EGR$Nmax.mean[i]+shift
  rosso(s_time[,i],EGRr[,i],C0r[,i],lag=0,Nmax)
}
f_concr=sapply(1:length(unique(r_time$group)),f_concfun)

#dimension of f_concr is runs x 13
##############################
#single distribution of doses
##############################

#pdf doses per population
doser_fun=function(i){
  #affect the portion size to the 13 RTE foods category
  #see table size in input file
  #in this table we have only 6 portion sizes in
   #column 3: Smoked fish
   #column 4: Gravad fish
   #column 5:Cooked meat
   #column 6:Sausage
   #column 7:Pâté
   #column 8:Soft and semi-soft cheese
   #here we need 13 columns respectively for:
     #Smoked fish ROP
     #Hot smoked fish    ROP
     #Gravad fish ROP
     #Cooked meat ROP
     #Sausage     ROP
     #Pâté  ROP
     #Cold smoked fish   normal
     #Hot smoked fish    normal
     #Gravad fish normal
     #Cooked meat normal
     #Sausage     normal
     #Pâté  normal
     #Soft and semi-soft cheese normal
  #the following duplicates the needed columns to have portion sizes
  #for all the RTE food categorie
  sizer=t((size[i,3:8]))
```

```
  sizer=c(sizer[1],sizer)
  sizer=c(sizer[-7],sizer)

  #dimension of dosei is runs x 13
  #the same is applied for the consumption (number of eating occasions)
  consoi=t((conso[i,3:8]))
  consoi=c(consoi[1],consoi)
  consoi=c(consoi[-7],consoi)
  consoi=consoi*ROP$p*ROP$p2
  consoi=consoi/sum(consoi)
  #dose is caculated
  dosei=t(t(f_concr)+log10(sizer))
  #dimension of dosei is runs x 13
  #ecdf function provide for each RTE food categries
  #the probability to observe a set of doses, see DoseCont definition
  ecdf_fun=function(j){
    x=dosei[,j]
    pp=ecdf(x)
    pCont1=pp((DoseCont-(step/2)))
    pCont2=pp((DoseCont+(step/2)))
    pdf=(pCont2-pCont1)
    pdf[1]<-1-sum(pdf[-1])
    pdf*consoi[j]
  }
  apply(sapply(1:13,ecdf_fun),1,sum)
}

#Overall prevalence per population
#for each food the function prev_fun calculate
#the overall prevalence
prev_fun=function(i){
  #determining the total eating occasion for the 13 RTE foods
  consoi=t((conso[i,3:8]))
  consoi=c(consoi[1],consoi)
  consoi=c(consoi[-7],consoi)
  #consoi is multiplied by p and p2 which reprenet respectively
  #p and p2 are extracted from the table ROP
  #p is proportion of ROP and normal pacjaging within eact RTE food category
  #p2 is proportion hot smoked and cold smoked
  #p2 is equal to 1 for the other food categories
  consoi=consoi*ROP$p*ROP$p2
  #consoi is now a proportion of consumption of one sub-category...
  consoi=consoi/sum(consoi)

  prevfood_fun=function(j){
    prev$S[j]/prev$N[j]*consoi[j]
  }
  #the overal prevalence is now calculated
  sum(sapply(1:13,prevfood_fun))
}
#derivation of the total number of eating oaccasions for all the RTF categories
teo=apply(conso[,3:8],1,sum)
#the overal prevalence is now calculated for each subpopulation
overall_prev=sapply(1:14,prev_fun)
overall_prev=data_frame(prev=overall_prev,Path=DRP$Path,
                        population=DRP$population,
                        teo)
#use of the pdf_dose function for all the subpopulations
pdf_dose=sapply(1:14,doser_fun)
#creating and organisation of a data-frame incuding the pdf of the doses
df_pdf_dose=data.frame(pdf_dose)%>%
  gather("population",prob,1:ncol(pdf_dose))

path=data_frame(population=unique(df_pdf_dose$population),Path=DRP$Path)
df_pdf_dose=left_join(df_pdf_dose,path,key=population)
df_pdf_dose=df_pdf_dose%>%
  group_by(Path)%>%
  mutate(cdf=cumsum(prob))
```

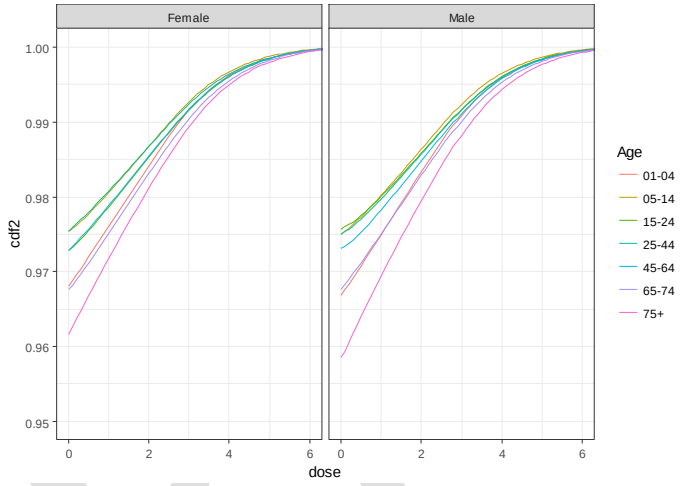
```
  #df_pdf_dose is a data frame where the vector DoseCont <- seq(0, 12, step) with
step=.1
  #is repeated 14 times (subpopulations) and for each dose we attribute its
probability...

  #df_pdf_risk contains the probability of listeriosis per case
  df_pdf_risk=df_pdf_dose
  #risk new variable in the dataframe is calculated by multiplying the probability
of a dose by
  #the conditional probability of listerioisis condititional to the same dose (see
script 1)
  df_pdf_risk$risk=df_pdf_dose$prob*df_DR$prob
  risk=df_pdf_risk%>%
    group_by(Path)%>%
    summarise(risk=sum(risk))
  risk=left_join(overall_prev,risk,key=Path)
  risk%>%
    mutate(risk=risk*prev,cases=round(risk*teo))
  # the output is a table with 14 lines (14 populations) including the overall
prevalence
  #risk per serving, teo, and the expected number of cases
}
####End contam function
```

In Figure 33 an example is given of the of simulated distribution of *L. monocytogenes* doses per eating occasion using the three options for the initial concentration of *L. monocytogenes* in the three RTE food subcategories.

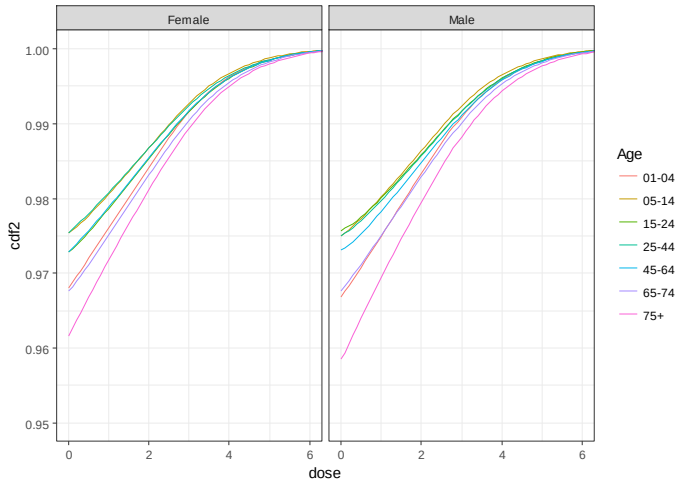
(a)



(b)



(c)



CDF: cumulative distribution function. (a) Option 1: using only the distributions estimated with BLS data; (b) Option 2: using only the distributions estimated with US data (Gombas et al., 2003); and (c) Option 3: using fish distribution from EU BLS data, and meat and cheese distributions from US data (Gombas et al., 2003).

Figure 33: Example of simulated doses distribution (log_{10} CFU of *L. monocytogenes* per eating occasions) using three options for the initial concentration of *L. monocytogenes* in three RTE food subcategories

Appendix D – Reported human cases of confirmed human listeriosis and notification rates in the EU/EEA, 2008–2015

6481 **Table 38:** Reported cases of confirmed human listeriosis and notification rates in the EU/EEA, by country and year, 2008–2015

Country	2008		2009		2010		2011		2012		2013		2014		2015	
	Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate
Austria	31	0.37	46	0.55	34	0.41	26	0.31	36	0.43	36	0.43	49	0.58	38	0.44
Belgium	64	0.60	58	-	40	0.37	70	-	83	0.75	66	0.59	84	0.75	83	0.74
Bulgaria	5	0.07	5	0.07	4	0.05	4	0.05	10	0.14	3	0.04	10	0.14	5	0.07
Croatia	-	-	-	-	-	-	-	-	0	0.00	0	0.00	4	0.09	2	0.05
Cyprus	0	0.00	0	0.00	1	0.12	2	0.24	1	0.12	1	0.12	0	0.00	0	0.00
Czech Republic	37	0.36	32	0.31	26	0.25	35	0.33	32	0.30	36	0.34	38	0.36	36	0.34
Denmark	51	0.93	97	1.76	62	1.12	49	0.88	50	0.90	51	0.91	92	1.62	44	0.78
Estonia	8	0.60	3	0.22	5	0.38	3	0.23	3	0.23	2	0.15	1	0.08	11	0.84
Finland	40	0.75	34	0.64	71	1.33	43	0.80	61	1.13	61	1.12	65	1.19	46	0.84
France	276	0.43	328	0.51	312	0.48	282	0.43	346	0.53	369	0.56	373	0.57	412	0.62
Germany	306	0.37	394	0.48	377	0.46	331	0.41	414	0.52	463	0.57	598	0.74	580	0.71
Greece	1	0.01	4	0.04	10	0.09	10	0.09	11	0.10	10	0.09	10	0.09	31	0.29
Hungary	19	0.19	16	0.16	20	0.20	11	0.11	13	0.13	24	0.24	39	0.39	37	0.38
Iceland	0	0.00	0	0.00	1	0.31	2	0.63	4	1.25	1	0.31	4	1.24	0	0.00
Ireland	13	0.29	10	0.22	10	0.22	7	0.15	11	0.24	8	0.17	15	0.33	19	0.41
Italy	118	0.20	109	0.18	157	0.27	129	0.22	112	0.19	143	0.24	132	0.22	153	0.25
Latvia	5	0.23	4	0.18	7	0.33	7	0.34	6	0.29	5	0.25	3	0.15	8	0.40
Lithuania	7	0.22	5	0.16	5	0.16	6	0.20	8	0.27	6	0.20	7	0.24	5	0.17
Luxembourg	1	0.21	3	0.61	0	0.00	2	0.39	2	0.38	2	0.37	5	0.91	0	0.00
Malta	0	0.00	0	0.00	1	0.24	2	0.48	1	0.24	1	0.24	1	0.24	4	0.93
Netherlands	45	0.27	44	0.27	72	0.43	87	0.52	73	0.44	72	0.43	90	0.53	71	0.42
Norway	34	0.72	31	0.65	22	0.45	21	0.43	30	0.60	21	0.42	29	0.57	18	0.35
Poland	33	0.09	32	0.08	59	0.16	62	0.16	54	0.14	58	0.15	87	0.23	70	0.18
Portugal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	28	0.27
Romania	0	0.00	6	0.03	6	0.03	1	0.00	11	0.05	9	0.04	5	0.03	12	0.06
Slovakia	8	0.15	10	0.19	5	0.09	31	0.57	11	0.20	16	0.30	29	0.54	18	0.33
Slovenia	3	0.15	6	0.30	11	0.54	5	0.24	7	0.34	16	0.78	18	0.87	13	0.63
Spain[(a)]	88	0.77	121	1.05	129	1.11	91	0.78	109	-	140	1.00	161	0.77	206	0.99
Sweden	60	0.65	73	0.79	63	0.67	56	0.59	72	0.76	93	0.97	125	1.30	88	0.90
United Kingdom	206	0.33	235	0.38	176	0.28	164	0.26	183	0.29	192	0.30	201	0.31	186	0.29
EU/EEA Total	1,459	0.35	1,706	0.42	1,686	0.42	1,539	0.36	1,754	0.42	1,905	0.45	2,275	0.49	2,224	0.48

- = No reported data. Source: ECDC Surveillance Atlas of Infectious Diseases, 19 April 2017 – Available at: http:/ /ecdc.europa.eu/en/data-tools/atlas/Pages/atlas.aspx#sthash.qAIHQymD.dpuf
(a): Sentinel system; estimated population coverage of 45% in 2014–2015, 30% in 2013 and 25% in 2009–2012.

Appendix E – Data reported in the EFSA zoonoses database on occurrence of strong-evidence food-borne outbreaks where *Listeria* spp. was the causative agent, 2008–2015

6484 **Table 39:** Reported strong-evidence food-borne outbreaks with *Listeria* spp. causative agent in the reporting countries from the EU in accordance with
6485 Directive 2003/99/EC[a] (2007–2014)

Food vehicle[b] (group)	Food vehicle[b]	Causative agent[c]	Serovar	Year	Country[d]	Extent[e]	Type of evidence[f]							Place of exposure[g]	Place of origin[h]	Contributory factor[i]	Food vehicle origin	Human cases	Hospitalisations[j]	Deaths[j]
							1	2	3	4	5	6	7							
Meat and meat products	Pigmeat and products thereof (sliced jellied pork)	*Lm*	4b	2008	AT	General	X	X	X					Restaurant or Cafe or Pub or Bar or Hotel or Catering service	Restaurant or Cafe or Pub or Bar or Hotel or Catering service	Cross-contamination	AT	14	7	0
Dairy	Cheese (cheese (acid curd) made from pasteurised milk)	*Lm*	1/2a	2009	DE	Unknown		X	X					Household	Unknown	NR	EU	6	6	2
Dairy	Cheese (acid curd cheese)	*Lm*	1/2a	2009	AT	General	X							Household	Processing plant	Cross-contamination	AT	25	25	5
Meat and meat products	Pigmeat and products thereof	*Lm*	1/2a	2009	CZ	General		X	X					Others	Others	Cross-contamination	CZ	9	9	4
Meat and meat products	Bovine meat and products thereof (beaf stew (sous vide))	*Lm*	unspecified	2009	DK	General	X								Processing plant	NR	NR	8	8	0
Meat and meat products	Other or mixed red meat and products thereof (tongue, beef, pork, ham, chicken, turkey)	*Lm*	1/2a	2010	UK	General		X	X	X				Disseminated cases	Processing plant	Cross-contamination	UK	10	10	2
Other	Other foods (salmon and cress sandwiches, Egg mayonnaise	*Lm*	O4	2010	UK	General		X	X	X				Hospital or medical care facility	Processing plant	Cross-contamination; Storage time/temperature abuse;Unproce	UK	4	4	1

Food vehicle[b] (group)	Food vehicle[b]	Causative agent[c]	Serovar	Year	Country[d]	Extent[e]	Type of evidence[f]							Place of exposure[g]	Place of origin[h]	Contributory factor[i]	Food vehicle origin	Human cases	Hospitalisations[j]	Deaths[j]
							1	2	3	4	5	6	7							
	sandwiches)															ssed contaminated ingredient				
Fish and seafood	Fish and fish products (herring casserole in vegetable oil)	*Lm*	4b	2010	DE	General		X	X					Household	Processing plant	Unknown	DE	12	8	1
Fish and seafood	Fish and fish products (gravad salmon)	*Lm*	unspecified	2010	DK	General				X				NR	NR	NR	NR	9	0	0
Dairy	Cheese	*Lm*	1/2a	2011	BE	General			X			X		Disseminated cases	Unknown	Cross-contamination	BE	11	11	4
Other	Mixed food (sandwiches various and prepared salad dishes)	*Lm*	O4	2011	UK	General				X				Hospital or medical care facility	Processing plant	Storage time/temperature abuse;Unprocessed contaminated ingredient	UK	3	3	0
Meat and meat products	Pigmeat and products thereof	*Lm*	1/2a	2011	CH	General			X			X		Household	Processing plant	Cross-contamination	EU	9	NR	0
Other	Bakery products (sponge cake)	*Lm*	NR	2011	FI	Household		X	X	X				Household	Processing plant	NR	Unknown	2	2	0
Other	Bakery products (pork pies)	*Lm*	4b	2012	UK	General				X				Disseminated cases	Processing plant	Cross-contamination	UK	14	14	1
Meat and meat products	Bovine meat and products thereof (pressed beef also called potted beef and beef stew)	*Lm*	1/2a	2012	UK	General				X				Mobile retailer or market/street vendor	Mobile retailer or market/street vendor	Cross-contamination	UK	4	4	2
Other	Mixed food (sandwiches)	*Lm*	unspecified	2012	UK	General			X			X		Hospital or medical care facility	Unknown	Other contributory factor	Unknown	6	6	2
Meat and meat products	Other or mixed red meat and products	*Lm*	NR	2012	FI	General			X	X		X		Hospital or medical care facility	Processing plant	Cross-contamination	FI	20	20	3

Food vehicle[b] (group)	Food vehicle[b]	Causative agent[c]	Serovar	Year	Country[d]	Extent[e]	Type of evidence[f]							Place of exposure[g]	Place of origin[h]	Contributory factor[i]	Food vehicle origin	Human cases	Hospitalisations[j]	Deaths[j]
							1	2	3	4	5	6	7							
	thereof (meat jelly)																			
Meat and meat products	Meat and meat products	*Lm*	NR	2013	SE	General	X							Disseminated cases	Unknown	Unknown	Unknown	34	NR	NR
Food of non-animal origin	Vegetables and juices and other products thereof (mixed salad)	*Lm*	4b	2013	DE	General		X	X			X		Hospital or medical care facility	Unknown	Unprocessed contaminated ingredient	DE	3	3	1
Fish and seafood	Crustaceans, shellfish, molluscs and products thereof (crab meat)	*Lm*	unspecified	2013	UK	General		X					X	Mobile retailer or market/street vendor	Processing plant	Cross-contamination	UK	4	4	1
Fish and seafood	Crustaceans, shellfish, molluscs and products thereof (crab meat)	*Lm*	unspecified	2013	UK	General		X					X	Mobile retailer or market/street vendor	Processing plant	Inadequate chilling	UK	3	3	1
Meat and meat products	Pigmeat and products thereof	*Lm*	1/2a	2013	BE	Household		X					X	Household	Farm	NR	NR	2	0	0
Dairy	Cheese	*Lm*	1/2b	2013	BE	Household		X					X	Household	Retail	Unprocessed contaminated ingredient	NR	2	0	0
Fish and seafood	Fish and fish products (half-fermented trout)	*Lm*	unspecified	2013	NO	General		X	X					Disseminated cases	Unknown	Unknown	NO	3	3	1
Fish and seafood	Crustaceans, shellfish, molluscs and products thereof	*Lm*		2013	FR	Household				X				Household	Unknown	Unknown	Unknown	3	1	0
Other	Mixed food (iceberg lettuce with yogurt dressing, gouda	*Lm*	1/2a	2014	DE	General				X				Hospital or medical care facility	Unknown	Unknown	DE	2	2	0

Food vehicle[b] (group)	Food vehicle[b]	Causative agent[c]	Serovar	Year	Country[d]	Extent[e]	Type of evidence[f]							Place of exposure[g]	Place of origin[h]	Contributory factor[i]	Food vehicle origin	Human cases	Hospitalisations[j]	Deaths[j]
							1	2	3	4	5	6	7							
	cheese)																			
Other	Other foods (cold cuts)	*Lm*[k]	NR	2014	DK	General	X	X	X			X	X	Others		Unknown		41	0	0
Other	Mixed food (composite meal)	*Lm*[k]	NR	2014	DK	General		X	X	X		X	X		Hospital or medical care facility	Unknown	NR	6	0	0
Fish and seafood	Fish and fish products (smoked trout and smoked halibut)	*Lm*[k]	NR	2014	DK	General		X	X			X	X	Others	NR	Unknown	NR	6	6	0
Meat and meat products	Other or mixed red meat and products thereof (sausage)	*Lm*	1/2a	2014	SE	NR		X	X					Disseminated cases	NR	NR	NR	4	NR	NR
Food of non-animal origin	Vegetables and juices and other products thereof (pre-cut salad)	*Lm*	4b	2014	CH	General		X	X					Household	Processing plant	Cross-contamination	CH	31	NR	4
Other	Buffet meals (sandwiches)	*Lm*	unspecified	2014	UK	General		X	X					Hospital or medical care facility	Hospital or medical care facility	Other contributory factor	Unknown	4	4	0
Other	Mixed food	*Lm*	4b	2015	PT	General		X	X	X		X		Hospital or medical care facility	Canteen or workplace catering	Cross-contamination	PT	3	3	0
Other	Mixed food (rice pudding)	*Lm*	4b	2015	DE	General		X	X	X				School or kindergarten	School or kindergarten	Storage time/temperature abuse	Unknown	159	2	0
Meat and meat products	Pigmeat and products thereof	*Lm*	1/2a	2015	IT	General	X	X	X	X		X		Multiple places of exposure in one country	Processing plant	Unprocessed contaminated ingredient, Cross-contamination	IT	12	12	2
Other	Mixed food (likely dill which then contaminated crustaceans and cheese)	*Lm*	4b	2015	SE	NR	X	X	X	X		X		NR	NR	NR	NR	13	1	NR
Other	Buffet meals	*Lm*	unspecified	2015	FI	General				X				Restaurant or	Restaurant or	Unprocessed	EEA	24	1	0

Food vehicle[b] (group)	Food vehicle[b]	Causative agent[c]	Serovar	Year	Country[d]	Extent[e]	Type of evidence[f]							Place of exposure[g]	Place of origin[h]	Contributory factor[i]	Food vehicle origin	Human cases	Hospitalisations[j]	Deaths[j]
							1	2	3	4	5	6	7							
														Cafe or Pub or Bar or Hotel or Catering service	Cafe or Pub or Bar or Hotel or Catering service	contaminated ingredient, Storage time/temperature abuse				

AT: Austria, BE: Belgium, CZ: Czech Republic, DK: Denmark, EEA: European Economic Area, EU: European Union, FI: Finland, FR: France, DE: Germany, NO: Norway, NR: not reported, PT: Portugal, SE: Sweden, CH: Switzerland, UK: United Kingdom.

(a): Food-borne outbreak: an incidence, observed under given circumstances, of two or more human cases of the same disease and/or infection, or a situation in which the observed number of human cases exceeds the expected number and where the cases are linked, or are probably linked, to the same food source (Directive 2003/99/EC).

(b): Food vehicle: Food (or foodstuff) that is suspected of causing human cases.

(c): Causative agent: The pathogen or its product, such as a toxin or bioactive amine, considered to be the cause of the food-borne outbreak.

(d): EU countries including Norway and Switzerland. Data from Spain have not been included in this table because they were provided outside the EFSA zoonoses database and in a different format of aggregation.

(e): Extent of outbreak: General outbreak: outbreak involving human cases from more than one household. Outbreaks in residential homes (e.g. nursing homes), schools and other similar institutions are considered to be general outbreaks. Household outbreak: outbreak where all the human cases live in one single household.

(f): Type of evidence: (1) Analytical epidemiological evidence: a statistically significant association between consumption of a foodstuff and being a case in an analytical epidemiological study (e.g. cohort or case–control study), (2) Detection in a food vehicle or its component: identification of the causative agent in a food vehicle or its component taken in the course of the investigation, (3) Detection in human cases: direct (e.g. culture) or indirect (e.g. serological) identification of the causative agent in clinical samples taken from outbreak cases, (4) Descriptive epidemiological evidence: suspicion of a food vehicle in an outbreak based on the identification of common food exposures, from the systematic evaluation of cases and their characteristics and food histories over the likely incubation period by standardised means (such as standard questionnaires) from all, or an appropriate subset of, cases, (5) Descriptive environmental evidence: e.g. evidence from food hygiene inspections, (6) Detection in food chain or its environment: identification of the causative agent in samples taken from the preparation or processing environment of the suspected food vehicle, or from batches of similar foodstuffs produced under the same conditions or in primary production where the suspected food vehicle originated, (7) Symptoms and onset of illness pathognomonic to causative agent.

(g): Place of exposure: this is the location ('setting') where the food was consumed or where the final stages of preparation of the food vehicle took place (e.g. cafe/restaurant, institution, home, takeaway outlet).

(h): Place of origin of problem: place where the contributory factors occurred.

(i): Contributory factor: fault or circumstance that singly or in combination led to the food-borne outbreak.

(j): The figure could be higher as for some outbreaks this was not reported.

(a): The database indicated *Listeria* spp., but the Annual Report on Zoonoses in Denmark 2014 (http://www.food.dtu.dk/english/publications) mentioned *L. monocytogenes*.

Appendix F – Overview of gene mutations in *Listeria monocytogenes* leading to a reduced virulence

6511 **Table 40:** Overview of important gene mutations in *L. monocytogenes* leading to a reduced virulence
6512 (reduced invasion, PI-PLC activity or cell-to-cell spread)

Source	Mutation-type	Gene targeted	AA position	Genetic lineage/serotype
Human, Food	*PMSC (type 2)*	*inlA*	656	I (1/2b)
Human	*PMSC (type 18)*	*inlA*	404	I (4b)
n. s.	*PMSC (type 16, 17)*	*inlA*	170, 253	I (1/2b)
Food	*PMSC (type 8, 10)*	*inlA*	460, 677	II (1/2a)
Human, Food, FPE	*PMSC (type 5, 7)*	*inlA*	189, 562	II (1/2a, 3a)
n. s.	PMSC (type 15)	*inlA*	77	II (1/2a)
Food	*PMSC (type 9)*	*inlA*	519	II (1/2c)
Human	*PMSC (type 14)*	*inlA*	539	II (1/2c, 3c)
Human, Food, FPE	*PMSC (type 3)*	*inlA*	700	II (1/2a, 3a; 3c)
Food, FPE	*PMSC (type 4)*	*inlA*	9	II (1/2a, 3a; 1/2c, 3c)
Human, Food	*PMSC (type 1)*	*inlA*	606	I (1/2b, 4b)+II (1/2a, 3a)
Human, Food	*PMSC (type 6)*	*inlA*	492	I (1/2b, 4b)+II (1/2a, 3a)
Human, Food, FPE	*PMSC (type 12)*	*inlA*	576	I (4b)+II (1/2c, 3c)
Food	*PMSC (type 11)*	*inlA*	685	I (1/2b)+II (1/2c)
Seafood	*PMSC (type 13)*	*inlA*	527	n. s.
Food (dairy products)	*Substitution (9bp)*	*inlB*	LRR-region	II (1/2a)
Food (dairy products)	*Substitution (12bp)*	*plcA*	17, 119, 262	II (1/2a)
Human	*Deletion (188bp)*	*brtA*	79	II (1/2c)
Bovine placenta (abortion)	*Deletion*	*prfA*	701	II (1/2a, 3a)
Pet food	*Deletion (1kb)*	*prfA*	n.s.	II (1/2a)
Human, Food	*Deletion (105bp)*	*actA*	n.s.	I (4a, 4b)

6513 PI-PLC: Phosphatidylinositol phospholipase C; AA: Amino acid; PMSC: Premature stop codon; LRR: Leucine-rich repeat region;
6514 n.s.: not specified.
6515 Source: (Roche et al., 2005; Temoin et al., 2008; Van Stelten and Nightingale, 2008; Van Stelten et al., 2010; Hain et al., 2012;
6516 Schwartz et al., 2012; Burall et al., 2014; Rupp et al., 2015).
6517

Appendix G – Summary statistics from the most recent surveys from the EFSA food consumption database

The summary statistics for the three RTE food categories sampled in the EU-wide BLS extracted from the EFSA food consumption database are provided in this Appendix. Tables 41 to 44 provide the summary statistics from the most recent surveys while Table 45 provides the comparison between two surveys in the same country for the age group 65–75 years old.

Table 41: Means of the median serving sizes (g) in the most recent (1997–2012 as starting date) national surveys from the EFSA food consumption database

Age groups (years)	Fish products				Meat products						Cheese	
	Gravad fish		Smoked fish		Cooked meat		Heat-treated sausages		Pâté		Soft and semi-soft cheese	
	F	M	F	M	F	M	F	M	F	M	F	M
1–4	25	-[(a)]	22	18	17	17	32	39	18	20	19	18
5–14	45	68	49	48	24	24	44	53	25	24	25	38
15–24	132	101	47	54	31	40	60	73	33	42	38	41
25–44	70	122	49	74	32	40	50	63	36	46	45	39
45–64	89	113	54	76	33	43	52	61	38	42	39	40
65–74	132	162	45	48	33	33	46	56	27	38	28	35
≥ 75	154	132	45	65	23	33	54	54	30	34	29	35

F: female; M: male.
(a): There were no servings in this group.

Table 42: Means of the 25th percentile of serving sizes (g) in the most recent (1997–2012 as starting date) national surveys from the EFSA food consumption database

Age groups (years)	Fish products				Meat products						Cheese	
	Gravad fish		Smoked fish		Cooked meat		Heat-treated sausages		Pâté		Soft and semi-soft cheese	
	F	M	F	M	F	M	F	M	F	M	F	M
1–4	20	-[(a)]	17	13	9	11	20	24	12	13	14	14
5–14	45	60	32	35	14	14	29	32	18	17	15	27
15–24	132	89	30	34	21	23	39	35	24	33	29	32
25–44	54	96	29	45	20	24	28	37	27	37	34	28
45–64	57	112	32	50	20	23	29	37	23	33	31	26
65–74	88	74	32	33	24	22	28	36	18	26	19	26
≥ 75	154	132	30	39	17	18	36	33	23	22	24	27

F: female; M: male.
(a): There were no servings in this group.

Table 43: Means of the 75th percentile of serving sizes (g) in the most recent national surveys (1997–2012 as starting date) from the EFSA food consumption database

Age groups (years)	Fish products				Meat products						Cheese	
	Gravad fish		Smoked fish		Cooked meat		Heat-treated sausages		Pâté		Soft and semi-soft cheese	
	F	M	F	M	F	M	F	M	F	M	F	M
1–4	30	-[(a)]	32	25	29	32	51	56	23	28	27	24
5–14	45	75	72	73	38	39	69	84	33	38	37	55
15–24	132	113	81	77	47	67	89	125	46	60	47	53
25–44	96	211	89	104	55	67	78	104	51	64	57	57
45–64	148	156	85	121	53	69	81	99	55	62	57	57
65–74	180	165	79	74	49	50	72	91	39	58	41	49
≥ 75	154	132	59	84	40	58	83	79	38	52	44	47

F: female; M: male.
(a): There were no servings in this group.

Table 44: Mean number of servings per person and year in the EU/EEA based on the mean number of servings per day estimated from the most recent national surveys (1997–2012 as starting date) in the EFSA food consumption database

Age groups (years)	Fish products				Meat products						Cheese	
	Gravad fish		Smoked fish		Cooked meat		Heat-treated sausages		Pâté		Soft and semi-soft cheese	
	F	M	F	M	F	M	F	M	F	M	F	M
1–4	0.63	0	26	28	71	78	95	88	56	59	22	18
5–14	0.49	0.19	8.3	8.0	93	99	92	101	36	43	18	17
15–24	1.5	2.4	14	8.8	98	135	58	91	24	35	24	20
25–44	3.6	2.3	12	13	121	159	67	108	24	41	33	29
45–64	4.6	4.5	19	22	127	164	73	114	22	39	34	36
65–74	10	7.9	37	42	141	168	75	101	29	45	38	44
≥ 75	3.0	2.0	55	87	123	153	70	110	43	65	46	65

F: female; M: male.

Table 45: Estimated change in the mean number of servings per person and year for the age group above 65 years old between two survey times for ready-to-eat (RTE) food

Gender		Change in mean no of servings per person and year			
		Denmark	Finland	The Netherlands	Sweden
Females	Cooked meat	117	14	-42	114
Females	Heat-treated sausages	147	-66	-12	-24
Females	Pâté	174	-5	-3	-143
Females	Smoked fish	-5	-6	-7	-5
Females	Gravad fish	ND	ND	ND	7
Females	Soft and semi-soft cheese	123	22	2	22
Females	Mean (all food groups)	111	-8	-12	-5
Males	Cooked meat	114	15	-16	134
Males	Heat-treated sausages	257	-85	3	-7
Males	Pâté	197	-5	4	-35
Males	Smoked fish	117	7	7	-16
Males	Gravad fish	ND	ND	ND	4
Males	Soft and semi-soft cheese	191	13	5	40
Males	Mean (all food groups)	175	-11	1	20

Red cells mark an increase, green cells mark a decrease and yellow cells mark small changes (– 5 to + 5). ND: no data.

Appendix H – Growth, survival and inactivation of *Listeria monocytogenes* in food and the food chain

Listeria monocytogenes growth/no-growth models

Based on microbial responses, expressed as changes in numbers and/or stress tolerance, the combinations of intrinsic and extrinsic environmental determinants to which microorganisms may be exposed, are divided into the domain of growth and the domain of no-growth which is associated with survival and/or death (inactivation) of microorganisms (Booth, 2002). The conditions that lie between these two domains refer to a zone where microbial responses are uncertain and characterised by the growth/no-growth interface (Le Marc et al., 2005). This zone is strongly associated with the so-called cardinal values (T, pH, a_w, etc.) for growth which outlines the bio-kinetic range of microbial proliferation. Such values are species- or even strain-dependent and thus, introduce significant variability in the assessment of the impact of marginal growth conditions on microbial growth, a common issue encountered in quantitative microbiological risk assessment. For instance, van der Veen et al. (2008) demonstrated variability in pH growth limits of 138 strains of *L. monocytogenes* at various temperatures from 7 to 46°C, at high salt level and in the presence of sodium lactate. To address growth limit variability in predictive modelling, mathematical models have been proposed that include theoretical growth limiting, cardinal values for critical hurdles, such as temperature, a_w, pH, $\%CO_2$ and preservatives, as biological meaningful parameters expressed either deterministically as fixed values or stochastically as probability distributions (Sanaa et al., 2004; Ostergaard et al., 2015).

The available probability (growth/no-growth) models for *L. monocytogenes* are commonly based on logistic regression (Table 46). They include polynomial expressions (Koutsoumanis et al., 2004; Mataragas et al., 2006; Gysemans et al., 2007; Skandamis et al., 2007; Vermeulen et al., 2007) or nonlinear equations (Tienungoon et al., 2000; Le Marc et al., 2005) with cardinal parameters, that describe the impact of growth controlling factors, such as temperature, pH, sodium chloride, organic acids and preservatives, on the probability of growth through a *logit* function. Assessment of the probability of growth in meat, dairy and seafood products has also been carried out through new growth models based on the gamma concept with interaction terms, as detailed in subsequent paragraphs. A few alternative (not based on logistic regression) cardinal growth/no-growth models are also detailed in Table 46. For instance, model #12 is based on the assumption that the minimum (cardinal) values for growth as well as the minimum inhibitory concentration (MIC) of preservatives are not independent of the other growth conditions in the food, suggesting the existence of synergistic effects).

Growth models

The most common secondary model types for predicting the microbial growth rate in responses to multiple combined inhibitors are the polynomial models (Dussault et al., 2016), the expanded square root models and the models based on the gamma concept, the latter two which are cardinal parameter models (CPM).

A recent version of polynomial models included sodium nitrite (0–200 ppm), pH (5.53–6.30), sodium chloride (0.51–1.85%), sodium acetate (0–0.74%), sodium lactate syrup (0–2.05%), calcium propionate (0–0.20%) and a blend of nisin and hop alpha acids (0–13.6 ppm) as predictor variables for the growth rate of *L. monocytogenes* in ham as a model of RTE meat products (Dussault et al., 2016).

The basic idea behind CPMs is to use model parameters that have a biological and/or graphical interpretation. This has the advantage that appropriate starting values are easy to determine when models are fitted to experimental data by nonlinear regression. In addition the models may be easily adjusted to account for different pathogen-food combinations by introducing the cardinal values and the maximum specific growth rate at optimum conditions (μ_{opt}) of the organisms in the target (e.g. new) food. Given that cardinal values may vary with strain, strain variability can be incorporated into the relevant models by replacing fixed point values with distributions, thereby converting the initial deterministic model into a stochastic one (Ostergaard et al., 2015).

6591 **Table 46:** Secondary cardinal models for the growth rate and growth/no-growth interface of *Listeria monocytogenes* in response to growth factors
6592 (temperature, pH and water activity) and growth inhibitors (organic acids; nitrites; phenolic compounds; carbon dioxide)

#	Equation	Type of model and information about the model on : (i) range of independent variables, (ii) use of single or multiple strains, (iii) outcome (probability of growth or growth rate)	Other comments	References
1	$$SR_n(X) = \begin{cases} 0, & X \le X_{min} \\ \left(\frac{X - X_{min}}{X_{opt} - X_{min}}\right)^n, & X_{min} \le X \le X_{opt} \end{cases}$$	Relative effect of growth factors ($T_{n=2}$, $pH_{n=1}$, $aw_{n=1}$) on μ_{max}. X_{min}, X_{opt} and X_{max}, are the minimal, optimum and maximum cardinal values, respectively	Typical square root model	(Zwietering et al., 1992; Gimenez and Dalgaard, 2004; Zuliani et al., 2007; Mejlholm et al., 2010) (Mejlholm and Dalgaard, 2007)
2	$$CM_n(X) = \frac{(X - X_{max})(X - X_{min})^n}{(X_{opt} - X_{\min})^{n-1}\{(X_{opt} - X_{min})(X - X_{opt}) - (X_{opt} - X_{max})[(n-1)X_{opt} + X_{min} - nX]\}}$$	Relative effect of T (n=2), pH (n=1) and a_w (n=2) on μ_{max}	-	(Rosso et al., 1995; Augustin et al., 2015) (Augustin and Carlier, 2000a)
3	$$SR\,(c) = \begin{cases} 1 - \left(\frac{c}{MIC}\right)^p, & c < MIC \\ 0, & c \ge MIC \end{cases}$$	Relative effect of growth inhibitors (NO_2, Phe, CO_2, organic acids, etc.) on μ_{max}	a:0.3 for potassium sorbate, 0.5 for acetate and diacetate, 1 for lactate and other inhibitors	(Zuliani et al., 2007; Mejlholm et al., 2010) (Augustin and Carlier, 2000a)
4	**a.** Model without interaction $$\mu_{max} = \mu_{opt}\, CM_2(T) CM_1(pH) CM_2(a_w) \prod_i^n SR(c_i) \prod_j^p k_{jl}$$ **b.** Models with interactions $$\mu_{max} = \mu_{opt} CM_2(T) CM_1(pH) CM_2(a_w) \prod_i^n SR(c_i) \prod_j^p k_{jl}\, \xi(T, pH, aw, c_i)$$ or	Cardinal growth model (gamma model) with or without interactions (ξ). -μ_{max} can be replaced by μ_{ref} corresponding to a reference temperature (T_{ref}) in the equation 1 above	*ξ: interaction term* *k_{jl}: l-th* level of the *j_{th}* corrective factor of the reference μ_{max} (defined as *level* 0 with k_0=1) for the impact of biotic (competitive microbiota) or abiotic (matrix structure,	(Le Marc et al., 2002; Gimenez and Dalgaard, 2004; Augustin et al., 2005; Zuliani et al., 2007; Mejlholm et al., 2010) (Augustin and Carlier, 2000b)

	$\mu_{max} = \mu_{opt} CM_2(T) CM_1(pH) SR_1(a_w) \prod_i^n SR(c_i) \prod_j^p k_{jl}\, \xi(T, pH, aw, c_i)$	-μ_{max} may be square root-transformed	agitation, fat, diffusion limitations, etc.)	(Mejlholm and Dalgaard, 2007)
5	$\xi = \begin{cases} 1, & \psi \le 0.5 \\ 2(1-\psi), & 0.5 < \psi < 1 \\ 0, & \psi \ge 1 \end{cases}$ with $\psi = \sum_i \frac{\varphi_{e_i}}{2\prod_{j \ne i}(1-\varphi_{e_i})}$	Formulas for calculation of interaction term in the cardinal models with interaction (*#4b*)	ψ value of 1 corresponds to the growth/no-growth interface	(Le Marc et al., 2002)
6	$\varphi_T = (1-\sqrt{\gamma(T)})^2;\ \varphi_{pH} = (1-\gamma(pH))^2;\ \varphi_{OA} = (1-\gamma(OA))^2$		γ(X): the relative effect of a single growth factor or inhibitor on μ_{max}	(Le Marc et al., 2002)
7	$\varphi_X = \left(\frac{X_{opt} - X}{X_{opt} - X_{min}}\right)^3, \varphi(NO_2, Phe, CO_2) = 1 - SR(NO_2) SR(Phe) SR(CO_2)$		*X* represents any of the growth factors *T, pH,* or *aw*	(Augustin et al., 2005)
8	$\varphi(T) = \left[1 - \frac{(T - T_{min})}{(T_{ref} - T_{\min})}\right]^2;\ \varphi(a_w) = \left[1 - \sqrt{\frac{(aw - aw_{min})}{(aw_{opt} - aw_{\min})}}\right]^2$ $\varphi(pH) = \left[1 - \sqrt{1 - 10^{(pH_{min} - pH)}}\right]^2;\ \varphi(Phe) = \left[1 - \sqrt{\frac{(Phe_{max} - Phe)}{Phe_{\max}}}\right]^2$ $\varphi(NO_2) = \left[1 - \frac{NO_{2_{max}} - NO_2}{NO_{2_{max}}}\right]^2;\ \varphi(CO_2) = \left[1 - \sqrt{\frac{(CO_{2_{max}} - CO_{2_{equilibrium}})}{CO_{2_{\max}}}}\right]^2$ $\varphi([LAC], [DAC], [AA]) = \left\{1 - \left[\left(1 - \sqrt{\frac{LAC_u}{MIC_{Und.lactic\ acid}}}\right).\left(1 - \sqrt{\frac{DAC_u}{MIC_{Und.diacetate}}}\right).\left(1 - \sqrt{\frac{AAC_u}{MIC_{Und.acetic\ acid}}}\right)\right]\right\}^2$			(Mejlholm et al., 2010) (Mejlholm and Dalgaard, 2007)
9	$\sqrt{\mu_{max}} = b(T - T_{\min})$	Square root model T: 7–30°C 3 strains	Minimally processed lettuce under MAP (5% O_2:15% CO_2:80% N_2)	(Sant'Ana et al., 2012)

10	$\sqrt{\mu_{max}} = b(T - T_{\min})$	Square root model T: 4–25°C 4 strains	Cut cantaloupe Honeydew Watermelon Aerobic storage	(Danyluk et al., 2014)
		T: 4–43°C 3 strains	Cut cantaloupe	(Fang et al., 2013)
11	$\sqrt{\mu_{max}} = b(T - T_{\min})$	Square root model T: 5–25°C 6 strains	Iceberg lettuce	(Koseki and Isobe, 2005)
12	$\sum_{i}^{n}\left[\frac{(X_{opt} - X)}{(X_{opt} - X_{min})}\right]^{3} = \prod_{j}^{k}(1 - \frac{c_i}{MIC})$	Growth/no-growth model: It defines the surface that delimits the growth area	X: growth factors (T, pH, or a_w) c_i: concentration of growth inhibitors. The limiting function of certain inhibitors may be expressed with shape parameter as in #3	(Augustin and Carlier, 2000b)
13	Logit P = $Ln(p/(1-p))=b_o+b_1ln(T-T_{min})+b_2ln^2(T-T_{min})+b_3ln[1- exp[0.536(T-48)]]+b_4ln(a_w-a_{wmin})+b_5ln(1-10^{pHmin-pH})+b_6ln^2(1-10^{pHmin-pH})$	Growth/no-growth model: Square and cross-terms may be included Two strains separately	Cardinal parameters fitted with non-linear logistic regression. If cardinal values are fixed then b_0-b_6 are estimated with linear logistic regression	(Tienungoon et al., 2000; Le Marc et al., 2005)
14	Logit $P = a_0+a_1T+a_2T^2+a_3pH+a_4pH^2+a_5sqrt(1-aw)+a_6(1-aw)+a_7*TpH+a_8Tsqrt(1-aw)+a_9pHsqrt(1-aw)$	Growth/no-growth model: T (4–30°C), pH (4.24–6.58) and aw (0.900–0.993) Agar versus broth Composite of strains	Ordinary logistic regression	(Koutsoumanis et al., 2004)
15	Logit $P = a_0+a_1T+a_2T^2+a_3SL+a_4SL^2+a_5SD+a_6SD^2+a_7*TSL+a_8TSD+a_9SLSD$	Growth/no-growth model: Aerobic versus anaerobic conditions. T (4 to 30°C), SL: 0 to 6% (vol/vol), and SD: 0 to 0.5% (wt/vol) with 0.5% or 2.5% NaCl Composite of strains	Ordinary logistic regression	(Skandamis et al., 2007)

16	Logit P = f(aw, pH, Lactic acid, contamination level)	Growth/no-growth model: Quantifies the growth potential of *L. monocytogenes* during the first 8 h of cheese-making at 30°C pH (5.6 to 6.5), a_w (0.938 to 0.96)	Polynomial expressions	(Schvartzman et al., 2010; Schvartzman et al., 2011)
17	P(T, pH, aw) = p(T)·p(pH)·p(aw), where $p(T) = \frac{\exp\left(\frac{T}{c}\right) - \exp\left(\frac{T_{inf}}{c}\right)}{\exp\left(\frac{T_{sup}}{c}\right) - \exp\left(\frac{T_{inf}}{c}\right)}$ $p(pH) = \frac{\exp(-pH) - \exp(-pH_{inf})}{\exp(-pH_{sup}) - \exp(-pH_{inf})}$ $p(a_w) = \frac{\mathrm{aw} - aw_{inf}}{\mathrm{aw}_{sup} - aw_{inf}}$	Growth/no-growth model: Probability of single cell growth T:5-25°C pH_{HCl}: 4.4–6.5 aw_{NaCl}:0.919–0.989 Single strain	T_{inf}, pH_{inf} and aw_{inf}: values below which, no growth occurs (P=0) T_{sup}, pH_{sup} and aw_{sup}: values above which, growth occurs with P=1 c: inflection point for the impact of temperature on P for growth	(Augustin and Czarnecka-Kwasiborski, 2012)

a_w: water activity; CO_2: carbon dioxide; MAP: modified atmospheric packaging, MIC: minimum inhibitory concentration, NO_2: nitrites; OA: organic acids; Phe: phenolic compounds; SL: sodium lactate, SD: sodium diacetate; T: temperature.

In general, it has been recommended that variability should be quantitatively expressed in risk estimates to the greatest scientifically achievable extent (WHO and FAO, 2007). An assumption frequently made by food microbiologists is that strain-to-strain variation of microbial behaviour is equal to or smaller than the experimental variation, and, as such, is not necessary to be determined and characterised (Whiting and Golden, 2002). Nevertheless, intra-species variability of microbial behaviour may have an important impact on the accuracy of microbiological risk assessment outcomes (Delignette-Muller and Rosso, 2000).

Strain variability

The inherent differences among identically treated strains of the same species, referred to as 'strain variability,' constitute an important source of variability in microbiological studies (Whiting and Golden, 2002). The variability of the growth kinetic behaviour among *L. monocytogenes* strains has been demonstrated in several studies (Rosenow and Marth, 1987; Junttila et al., 1988; Walker et al., 1990). Barbosa et al. (1994) compared 39 *L. monocytogenes* strains with respect to their growth potential at 4, 10 and 37°C, and demonstrated a highly strain-dependent growth behaviour of the pathogen as evaluated based on the estimated values of lag phase, exponential growth rate and generation time. Growth differences among four strains of the organism were also documented in vacuum-packaged ground beef of normal or high pH stored at 4°C (Barbosa et al., 1995). Avery and Buncic (1997) reported that clinical *L. monocytogenes* isolates exhibited on average a shorter lag phase compared to meat isolates in culture broth at 37°C, a difference which was even more evident when cultures were previously stored at 4°C under starvation. When growth of 58 *L. monocytogenes* strains was evaluated in meat broth under different combinations of temperature (10 or 37°C), pH (5.6 or 7.0) and a_w (0.960 or 1.00), the observed strain variability of the estimated lag phase was up to a factor of 25 and in growth rates up to a factor of three under the tested conditions (Begot et al., 1997). The findings of subsequent investigations characterising the growth behaviour of *L. monocytogenes* were similar with regard to strain variability (Buncic et al., 2001; De Jesus and Whiting, 2003; Uyttendaele et al., 2004; Lianou et al., 2006). For instance, De Jesus and Whiting (2003) characterised 21 *L. monocytogenes* strains with respect to their growth behaviour in culture broth (pH 6.5 and 0.1 M lactate) at 5 or 35°C, and reported considerable strain and, in some cases, intra-lineage variation; at 5°C, the estimated lag phase values ranged from 0.9 to 4.83 days and growth rate values from 0.33 to 0.59 log units per day. Similarly, as reported by Uyttendaele et al. (2004), the response of *L. monocytogenes* to suboptimal growth conditions in culture broth (at different combinations of temperature, pH, a_w, and NaCl and sodium lactate concentrations) was shown to be strain dependent, while strain variability was also observed when growth of selected strains was evaluated in modified broth simulating conditions associated with cooked ham or pâté. In general, the growth variability among strains of *L. monocytogenes* appears to increase at growth conditions, and particularly temperatures, away from the optimum for this organism, or otherwise close to the growth boundaries (Barbosa et al., 1994; Begot et al., 1997; Lebert et al., 1998; De Jesus and Whiting, 2003; Lianou et al., 2006).

With the newly proposed CPM with interactions (#4b in Table 46), both the growth rate and the growth/no-growth interface of *L. monocytogenes* could be predicted **simultaneously** by identifying those combinations of growth factors (e.g., pH, a_w and T) that result in a psi value ("*ψ*") equal to 1 or higher. The latter is involved in the calculation of the of the model interaction term ("ξ") equal to 1 or higher. This concept may be applicable to a variety of foods. The most updated version of the above model (#4b in Table 46) was presented by Mejlholm et al. (2010), using the values of 1.168, 0.565, 1.168 and 0.742/h for the μ_{opt} of meat, seafood, poultry and dairy products, respectively, by the paper of Augustin et al. (2005).

Figure 34 shows the impact of strain variability for the growth/no-growth interface and that some strains are capable of growing also at psi-values greater than one. These effects are more pronounced at 10 than at 4°C. In comparison, the grey shaded area in the figure indicates pH and a_w combinations defined in the Regulation (EC) No 2073/2005 as conditions that do not allow growth of *L. monocytogenes*. This comparison illustrates the importance of taking strain and storage temperature variability as well as model uncertainty into consideration when defining no-growth conditions.

Cardinal models are easily expandable to account for variability and for the increasing number of factors influencing microbial growth, especially organic acids which are naturally occurring (e.g., lactic acid) or added as preservatives (e.g., organic acid salts). Tables 47, 48 and 49, list different reported MIC, optimum specific growth rates and other cardinal parameter values as an illustration on the variability and types of available data.

The inhibitory effect of organic acids is mainly attributed to its undissociated molecule and to some extent to acidification (pH-reducing potential). That is why the effect increases at low pH. The MIC values of *L. monocytogenes* to various organic acids is shown to be strain- and pH-dependent, especially close to the growth limiting pH (e.g. <4.8), with the highest observed variation, being almost 9.0 mM (Wemmenhove et al., 2016). The average MICs of undissociated lactic, acetic, citric, and propionic acid were 5 ± 1.5 mM, 19.0 ± 6.5 mM, 3.8 ± 0.9 mM, and 11.0 ± 6.3 mM, respectively, for six *L. monocytogenes* strains tested in a pH range of 5.2 to 5.6. The magnitude of MIC in the latter pH range was a little higher than that at pH 4.6.

Table 47: Summary or reported minimum inhibitory concentrations of compounds that may inhibit growth of *Listeria monocytogenes* and that have been proposed for use in cardinal models

Compound	MIC	Comments – additional information	Reference
Lactic acid	5.40 mM	Median	(Augustin and Carlier, 2000a)
	3.79 mM	Estimated	(Mejlholm et al., 2010)
	1.76 mM	Estimated at 20°C	(Zuliani et al., 2007)
	8.00 mM	Estimated 20°C	(Le Marc et al., 2002)
Acetic acid	20.1 mM	Median	(Augustin and Carlier, 2000a)
	10.3 mM	Estimated	(Mejlholm et al., 2010)
	5.83 mM	Estimated at 20°C	(Zuliani et al., 2007)
	20.3 mM	Estimated at 20°C	(Le Marc et al., 2002)
Propionic acid	8.8 mM	Estimated at 20°C	(Le Marc et al., 2002)
Citric acid	1.6 mM	Median	(Augustin and Carlier, 2000a)
Potassium sorbate	5.1 mM	Single	(Augustin and Carlier, 2000a)
	4.31 mM	Mean	(Zuliani et al., 2007)
Sodium benzoate	0.7 mM	Single	(Augustin and Carlier, 2000a)
Sodium diacetate	4.8 mM	Estimated at 8°C	(Mejlholm and Dalgaard, 2009)
	0.25%	At 4°C, 2.5% NaCl, anaerobic conditions	(Skandamis et al., 2007)
	0.3-0.5%	At 4°C and 0.5%, or aerobic conditions, or >4°C	
NO_2	11.4 µM	Median	(Augustin and Carlier, 2000a)
	25.0 µM	Mean	(Augustin et al., 2005)
	7.61 µM	Equals to 350 ppm	(Mejlholm et al., 2005)
	54.2 µM	Median	(Augustin and Carlier, 2000b)
Sodium lactate	5.95%	Fitted	(Devlieghere et al., 2001)
	4-5%	At 4°C, 2.5% NaCl, anaerobic conditions	(Skandamis et al., 2007)
	>6%	At 4°C and 0.5%, or aerobic conditions, or >4°C	
Phenol	31.9 ppm	Fitted (smoked salmon)	(Augustin et al., 2005)
	28.1 ppm		(Gimenez and Dalgaard, 2004)
			(Mejlholm and Dalgaard, 2007)
	12.5 ppm		(Augustin and Carlier, 2000a)
	32.0 ppm		(Mejlholm et al., 2010)
CO_2	3.04%	Partial pressure of CO_2 above atmospheric	(Augustin et al., 2005)
	1.64%	Proportion	(Augustin and Carlier, 2000a)
	5.08%	Proportion	(Augustin and Carlier, 2000b)
	3,140 ppm	Dissolved CO_2 at equilibrium	(Mejlholm and Dalgaard, 2007); (Mejlholm et al., 2010)

MIC: minimum inhibitory concentration.

6665 **Table 48:** Reported optimum specific growth rates (h^{-1}) used in cardinal growth models

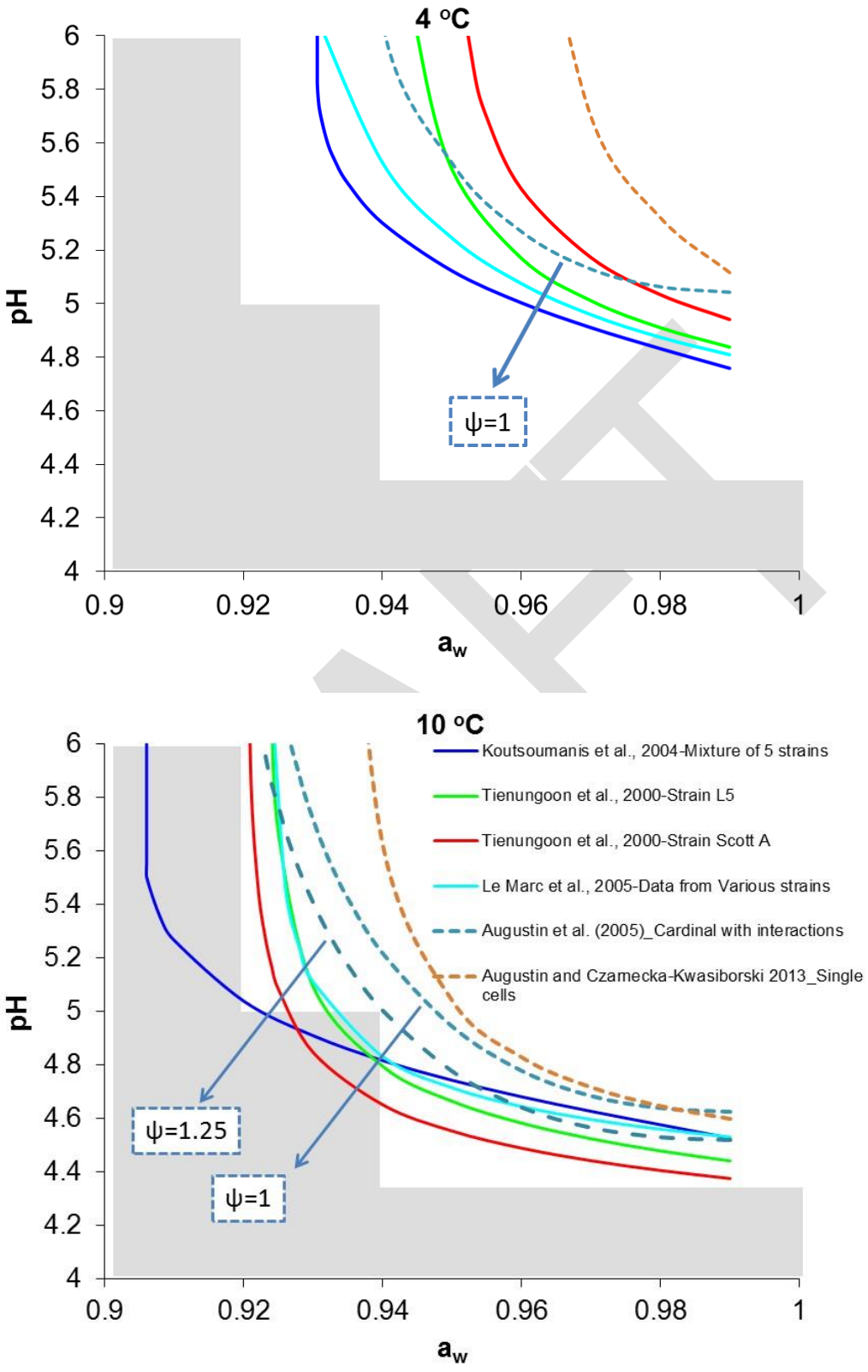
Cardinal parameter	Value	Concomitant variables or associated foods	Reference
μ_{opt}	1.14	30°C, pH 7.0	(Le Marc et al., 2002)
μ_{opt}	0.85	pH 7.1, T_{opt}=37°C, a_w=0.997	(Zuliani et al., 2007)
μ_{opt}	0.700	Dairy	(Augustin and Carlier, 2000a)
	1.318	Meats	
	1.061	Seafoods	
μ_{opt}	0.742	Dairy	(Augustin et al., 2005)
	1.168	Meat	
	0.565	Seafoods	
μ_{ref}	0.419	Seafood at 25°C	(Mejlholm et al., 2010)
	1.056	Median	(Augustin and Carlier, 2000b)

6666 a_w: water activity.

6667 **Table 49:** Reported cardinal values for growth of *Listeria monocytogenes*

Parameter	Value	Additional information	Reference
T_{min}	-2.0	Fitted	(Le Marc et al., 2005)
	-2.7	Median	(Augustin and Carlier, 2000b)
	-3.0	Median	(Augustin and Carlier, 2000b)
	-1.72	Median	(Augustin et al., 2005)
	-4.5	Fitted	(Le Marc et al., 2002)
	-2.83	Fitted (seafood)	(Mejlholm et al., 2010)
	-2.3	Fitted (seafood)	(Mejlholm and Dalgaard, 2007)
	-1.623[(a)];0.4164[(b)]	Fitted	(Tienungoon et al., 2000)
T_{opt}	37		All sources
	37.4	Fitted	(Le Marc et al., 2002)
T_{max}	45.5		All sources
pH_{min} (acetic acid)	4.79	Median	(Augustin and Carlier, 2000b)
pH_{min} (lactic acid	4.54	Median	(Augustin and Carlier, 2000b)
	4.71	Mean	(Augustin et al., 2005)
	4.97	Fitted	(Mejlholm et al., 2010)
pH_{min} (citric acid)	4.37	Median	(Augustin and Carlier, 2000b)
pH_{min} (propionic acid)	5.0	Median	(Augustin and Carlier, 2000b)
pH_{min} (malic acid)	4.4	Median	(Augustin and Carlier, 2000b)
pH_{min} HCl	4.38	Median	(Augustin and Carlier, 2000b)
	4.26	Mean	(Augustin et al., 2005)
	4.21	Fitted	(Le Marc et al., 2002)
	4.20	Fitted	Le Marc et al. (2005)
	3.350	Fitted	(Tienungoon et al., 2000)
pH_{min} (various studies)	4.55	Median	(Augustin and Carlier, 2000a)
pH_{opt}	7.1		All sources
	7.21	Fitted	(Le Marc et al., 2002)
pH_{max}	9.61	Median	(Augustin and Carlier, 2000a)
pH_{max}	9.61	Median	(Augustin and Carlier, 2000a)
	10.07	Fitted	(Le Marc et al., 2002)
$a_{w, min}$ (NaCl)	0.915	Fitted	(Le Marc et al., 2005)
	0.914	Fitted	(Tienungoon et al., 2000)
	0.913	Mean	(Augustin et al., 2005)
	0.910	Median	(Augustin and Carlier, 2000a)
$a_{w, min}$ (glycerol)	0.888	Median	(Augustin and Carlier, 2000b)
$a_{w, min}$ (sucrose)	0.918	Median	(Augustin and Carlier, 2000b)
$a_{w, min}$ (propylene glycol)	0.930	Median	(Augustin and Carlier, 2000b)
$a_{w, min}$ (drying)	0.949	Median	(Augustin and Carlier, 2000b)
$a_{w, min}$	0.923	Fitted	(Mejlholm et al., 2010)
$a_{w, opt}$	0.997	Arbitrary	(Augustin and Carlier, 2000a)
$a_{w, max}$	1.000	Mean	(Augustin and Carlier, 2000a)

6668 (a): strain L5.
6669 (b): strain Scott A.



Note: Psi-values greater than 1 indicate the predicted no-growth zone based on a cardinal model with interactions (Augustin et al., 2005). Shaded area indicate pH and a_w combination defined in Regulation (EC) No 2073/2005 as conditions that do not allow growth of *L. monocytogenes* (pH ≤ 4.4 or a_w ≤ 0.92, or pH ≤ 5.0 and a_w ≤ 0.94).

Figure 34: Reported growth/no-growth interfaces of *Listeria monocytogenes* at 4°C (upper) and 10°C (lower) with respect to pH and a_w, as predicted at a probability level of 0.1 by four different available models developed using different strains

Impact of food microflora on growth of *Listeria monocytogenes*

Most foods are complex with a heterogeneous microbial population. In the natural pursuit of growth and survival, interactions with either negative, neutral or positive effects on growth and survival may occur between different strains and species. For instance, *L. monocytogenes* present a different growth behaviour when inoculated in sterile meat than in natural contaminated meat (Marshall et al., 1992). In the latter case growth of pseudomonads stimulates this pathogen. The hydrolysis of proteins, which could provide free amino acids, has been considered as a likely explanation for the stimulus of *L. monocytogenes* growth by pseudomonads. Conversely, the growth of *L. monocytogenes* is known to be negatively affected by the competitive growth of lactic acid bacteria, naturally present as indigenous (spoilage) microbiota or added as starter or aroma cultures in dairy products (Ostergaard et al., 2014). The proposed mathematical approaches to model the interaction between lactic acid bacteria and *L. monocytogenes* are mainly based on the Jameson effect model or the Lotka-Volterra competition model (Cornu et al., 2011), which consider that the growth of the pathogen starts to be affected (retarded or even halted but rarely stimulated) as the population of lactic acid bacteria (or of the competitor in general) approaches a critical level, that is close to stationary phase of growth. Such an approach has been successfully applied to model *L. monocytogenes* growth in processed seafood, mayonnaise-based seafood salads pork products and cottage cheese, both at constant and fluctuation temperatures, deterministically and stochastically (Gimenez and Dalgaard, 2004; Cornu et al., 2011; Ostergaard et al., 2014; Mejlholm and Dalgaard, 2015).

Limited information is available about the relation between growth of pathogens (safety) and spoilage (shelf life) of foods. In most microbiological risk assessments published up till now spoilage is not taken into account. Ignoring spoilage, however, may lead to erroneous estimations of risk because conditions leading to critical levels of the hazard usually favour spoilage. For example, abusive storage temperatures increase the probability of high concentrations of a pathogen at the time of consumption but also reduce the probability of consumption since spoilage is more likely to occur and prevent consumer from being exposed by functioning as warning of unacceptable products (Koutsounianis, 2009). Thus, being able to consider the impact of products wasted by the consumers due to evident spoilage at the time of consumption, on the exposure to *L. monocytogenes* would lead to more realistic risk assessments.

Food structure and composition

The chemical composition and structure of food (e.g. liquid vs solid foods, planktonic growth vs surface growth) is a crucial determinant of microbial growth and survival.

Furthermore, the microstructure of the food matrix can affect the growth of a colony by imposing physical restraints on microorganisms, by limiting the diffusion of essential nutrients and oxygen or by preventing the diffusion of metabolic products (Robins and Wilson, 1994). Microbial growth in liquid laboratory media, in which most of the existing models have been developed, can differ significantly from growth on a solid food since in the latter the rates of diffusion of molecules are lower, the nutrients around a microcolony are utilised rapidly and not quickly replaced, while metabolites diffuse away slowly from the colony. If bacteria are suspended in liquids, their growth is planktonic and the motility of microorganisms may enable taxis to certain nutrient-rich sites of the food (Wilson et al., 2002).

Growth and survival of pathogens in foods can also be different than growth on meat surface due to oxygen availability while fat concentration is an additional parameter that may affect microbial behaviour on meat products. If bacteria are growing in structured aqueous phase, e.g., due to addition of thickeners, or gelling (structure-inducing) agents, such as gelatin, pectins, starch, gums, etc., microbial cells are immobilised within the gelled regions and constrained to grow as submerged colonies in three dimensions. Their growth rates as colonies tend to be lower than that of planktonically growing cells (Wilson et al., 2002; Theys et al., 2008; Aspridou et al., 2014; Boons et al., 2014; Skandamis and Jeanson, 2015). This can be further enhanced by increasing the fat concentration on the expense of water phase, thereby increasing the size of oil droplets.

If bacteria are growing on the surface of foods, such as meat and vegetables, growth is also colonial, initially in two dimensions (mono-layer), whereas the centre of colony gradually develops in the third dimension most likely upward, depending on aeration and nutrient availability (Skandamis and

Jeanson, 2015). Replenishment of nutrients takes place only from the bottom or the perimeter of the colony and soon cells in the centre of colony experience starvation and self-toxication. This places growth constraints to the surface colony as a whole and causes suppression of the growth rate as compared to submerged growth within the food matrix or planktonic growth.

Impact of stresses and shifts in the environment on lag time of *Listeria monocytogenes*

The number of models for the growth rate of *L. monocytogenes* is markedly higher than that for lag time. Lag time depends on current growth conditions and on cell 'history,' which defines the capacity of the organism to adapt and re-grow in the new environment.

In physiological terms, lag represents a transition period during which cells adjust to their new environment. Possible causes of lag could be change in nutrition, change in physical environment, presence of an inhibitor and state of the inoculum. Despite the numerical definition of the lag time as a time period (e.g., in min, h or days), from a biological (mechanistic) standpoint it represents the physiological state of cells (termed '*qo*' in the well-known Baranyi model; (Baranyi et al., 1995)) entering a new environment. The most common approach for introducing lag time in growth models is to describe it as a function of a unitless variable representing the adaptation work that is the work needed so that cells enter the exponential phase. This work has been termed '*work-to-be-done*' or '*relative lag time*' (also termed '*ho*' in the Baranyi model).

Many studies have demonstrated the effect of pre-incubation conditions (composition of the medium, temperature, pH, a_w etc.) on the lag duration of a number of pathogens and recent reports quantitatively describe the impact of up- and down- shifts in salinity and pH on the lag time of *L. monocytogenes* (Le Marc et al., 2010; Belessi et al., 2011b). Another situation that may strongly impact the physiological state of cells is their life within a biofilm. Detachment of such cells from the biofilm and translocation to a food (e.g., due to contamination) may be sensed as a shift in the environment and thus, induce lag time. Attached cells may be subjected to a metabolic repression that makes them behave more as stationary phase cells, when they are dislodged from surfaces, compared to those cells that had never been attached (Poimenidou et al., 2009; Belessi et al., 2011a).

Belessi et al. (2011b) reported that the lag time increased with osmotic downshifts, as well as by pH downshift from optimum to 5.1. Conversely, any type of shift within pH 5.5–7.2 did not markedly affect the lag times of *L. monocytogenes*. The longer the cells were incubated at no-growth a_w (0.90), the faster they initiated growth subsequently, suggesting adaptation to osmotic stress. Conversely, extended habituation at pH 4.9 had the opposite effect on subsequent growth of *L. monocytogenes*. These results suggest that there is an adaptation or injury rate induced at conditions inhibiting the growth of the pathogen. Therefore, exposure at no-growth conditions may also trigger adaptation phenomena, which could enhance or impair growth of the bacterium upon subsequent transfer to growth-supporting conditions.

Single cell heterogeneity

Population-wise, not all cells of a genetically homogeneous population are capable of initiating growth simultaneously when they experience a shift in growth conditions. This implies the existence of individual cell heterogeneity and suggests that a fraction of cells continues to grow unaffected by the shift (e.g., see the definition of the '*ao*' value of the Baranyi model), while the remaining population will gradually enter the exponential phase with the lag time of individual cells following probability distributions (Francois et al., 2006; Guillier and Augustin, 2006; Koutsoumanis and Lianou, 2013).

Traditional predictive microbiology uses deterministic mathematical models which describe the growth of large microbial populations as a whole without considering the variability in responses of individual cells. Koutsoumanis and Lianou (2013) showed that as a result of the heterogeneity in cell division time, growth of single cells or small microbial populations presents a high variability, and can be considered as a pool of events each one of which has its own probability to occur. In addition, the apparent variability in population growth gradually decreases with increasing the number of cells of this population at the beginning of incubation (time 0). A significant heterogeneity has been also observed in the ability of individual cells to initiate growth. Aguirre and Koutsoumanis (2016) showed that the a_w growth limits of *L. monocytogenes* individual cells varied from 0.940 to 0.997 and 0.951 to 0.997 for unheated and heat stressed cells, respectively. Due to the variability in the growth limits of individual cells, stressful conditions result in the presence of a non-growing fraction within the

bacterial population which results in a longer apparent lag time and an increased variability in the population growth.

The importance of single cell variability was raised after the recent developments in quantitative microbiological risk assessment. Deterministic models which provide point estimates are generally not sufficient to satisfactorily inform management of microbial safety risks. Indeed, if, for instance, the consequences of unacceptable levels of pathogenic microorganisms in a food are grave, knowledge only of the mean population growth is unlikely to be a sufficient basis for management decisions on the safety risk. Since contamination with pathogens usually occurs with very low numbers, the development of stochastic approaches that can describe the variability of single cell behaviour is necessary for realistic estimations of safety risks.

Modelling inactivation/survival of *Listeria monocytogenes*

Inactivation may be the result of heat (thermal) or non-thermal inimical factors, such as low pH (<4.0), low a_w (<0.90), high hydrostatic pressure or a lethal combination of those. Fewer inactivation models than growth models have been reported and in Table 50, an overview of the available inactivation models for *L. monocytogenes* is provided. Meta-analysis of existing (scattered) inactivation data over the last 20 years has assisted in modelling thermal inactivation of *L. monocytogenes* in various foods with different intrinsic properties and over a wide temperature range. van Lieverloo et al. (2013) investigated the thermal inactivation of *L. monocytogenes* in liquid food products by means of multiple regression models, taking into account 51 different strains of the pathogen and 6 cocktails of strains. The food products assayed were dairy (milk, cream, butter), fruit and vegetable juices, liquid eggs and meat gravy. The purpose of the work was to develop a model that could predict thermal inactivation of the pathogen while accounting for effects of food composition (pH, sodium chloride, sugar) and processing conditions (storage temperature, heat shock). The authors demonstrated that multiple regression modelling can be used effectively to predict the inactivation of the pathogen with a limited and realistic uncertainty level while retaining the variability of heat resistance observed among all strains assayed.

Recently, single or double Weibull inactivation models (Mafart et al., 2002; Coroller et al., 2006) have become increasingly popular. This is due to their capacity to fit all types of inactivation curves, including linear and non-linear, convex or concave, thus, describing curves with shoulder, tail and double inactivation phases. They are based on the alternative hypothesis that microbial inactivation is a cumulative form of a temporal distribution of lethal events that represent the spectrum of resistances of the treated microbial population to the lethal agent (Peleg and Penchina, 2000).

Since most RTE foods of concern for listeriosis are commonly contaminated post-processing, the non-thermal inactivation is an important trend that needs to be quantified in order to estimate the likely dose reaching the consumer in the context of QMRA.

Non-thermal inactivation

Non-thermal inactivation is usually the result of the single or combined effect of low pH (<4.5) or a_w (<0.90) and moisture (<60%) at refrigeration or ambient temperatures in the presence or not of preservative agents close to their minimum inhibitory concentration (MIC). Such conditions may be encountered in various RTE foods, mainly involving fermentation or ripening/drying, such as fermented meat and cheese. Although the lethality is attributed to heat-independent factors, temperature values within the bio-kinetic range of growth from the minimum (suboptimal, 0–5°C) to the maximum (super-optimal: 45–47°C) value for growth, remain the factor governing the non-thermal inactivation rate of bacteria (Shadbolt et al., 1999; Ross et al., 2008; McQuestin et al., 2009; Zhang et al., 2010b). The latter studies sufficiently demonstrated this concept for non-thermal inactivation of *E. coli* and *L. monocytogenes* at pH (3.5 to 5.1) and a_w (0.76 to 0.94) combinations commonly applying to various dry and fermented meats.

The work of Coroller et al. (2012) presents a modelling approach for non-thermal inactivation based on the gamma hypothesis that predicts the global behaviour of *L. monocytogenes* in various media. The proposed model postulates that only two microbial responses can be observed: growth or inactivation. When the maximum growth rate (as estimated from the gamma concept) is greater than zero, microbial growth is predicted. When the maximum growth rate is equal to zero, then the bacterial population is inactivated. The underlying principle is that growth, survival or inactivation of

microorganisms are time-dependent and it can be reasonably postulated that if the microbial behaviour was observed in static conditions for an infinite time period, only growth or inactivation would be observed. A microbial population would therefore be characterised by either slow growth or slow inactivation and the concept of infinite lag would have no meaning in this context. The environmental factors of interest are commonly temperature, pH, sodium chloride salt, a_w and commonly encountered organic acids such as sorbic acid, lactic acid and acetic acid. For further application in an industrial set up, the modelling approach of Coroller et al. (2012) had to meet the additional requirements which are described in Coroller et al. (2012).

6846 **Table 50:** Overview of thermal and non-thermal inactivation models for *Listeria monocytogenes*

#	Output (response variable)	Equation type	Model variables and ranges	Substrate/Food	Thermal or non-thermal	References
1	$Ln(t_{4D})$[a]	Quadratic or cubic polynomial expression	T: 4-42°C NaCl: 0.5-19% pH 3.2-7.3 Lactic acid: 0-2% w/w $NaNO_2$: 0-200 ppm Undissociated lactic acid Undissociated nitrous acid	BHI broth under reduced O_2 (100-150 ppm O_2) or under aerobic conditions	Non-thermal	(Buchanan and Golden, 1995; Buchanan et al., 1997)
2	Death rate	Gamma model including Bigelow terms	T: 0-43°C pH: 3.3-10.0 Sorbic acid: 0-0.3% Lactic acid: 0-18%	Data from Sym'previus, Combase and literature	Non-thermal (extending from growth to inactivation domain)	(Coroller et al., 2012)
3	Ln D	Quadratic expression	T: 55-65°C NaCl: 0.5-19% Sodium pyrophosphates: 0-0.3% w/w pH: 4-8.0	Beef gravy	Thermal	(Juneja and Eblen, 1999)
4	D-value	Quadratic expression	T: 57.5-62.5°C NaCl: 0-3% w/w Apple polyphenols: 0-3% w/w	Ground beef	Thermal	(Juneja et al., 2013)
5	D-value, z-value	Log-linear	T: 56-62°C	Fruit juices (apple, orange and grape)	Thermal	(Mazzotta, 2001a)
6	D-value, z-value	Log-linear	T: 58-66°C	Surimi-based imitation of crab meat	Thermal	(Mazzotta, 2001b)
7	D-value, z-value	Log-linear	T: 55-70°C	RTE chicken-fried beef patties	Thermal	(Osaili et al., 2006)

6847 D-value: the time for a one-log reduction at a constant temperature, Ln: natural logarithm, z-value: the temperature shift needed to change the D-value by one log-unit.
6848 (a): Natural logarithm of the time for 4 decimal (4 D) inactivation.

6849

Appendix I – Results from the outsourcing activity 2 risk assessment

6850 **Table 51:** Number of human listeriosis cases per million servings associated to the scenarios in RTE food subcategories

Scenarios	Population subgroups		
	Healthy	Elderly	Pregnant
Cold-smoked fish			
ROP/sliced	$5.74x10^{-4}$ ($4.36x10^{-4}$, $7.11x10^{-4}$)	$4.37x10^{-3}$ ($3.32x10^{-3}$, $5.42x10^{-3}$)	$1.27x10^{-1}$ ($9.68x10^{-2}$, $1.58x10^{-1}$)
ROP/non-sliced	$6.88x10^{-4}$ ($4.13x10^{-4}$, $1.05x10^{-3}$)	$5.24x10^{-3}$ ($3.15x10^{-3}$, $8.04x10^{-3}$)	$1.53x10^{-1}$ ($9.16x10^{-2}$, $2.34x10^{-1}$)
Normal/sliced	$4.19x10^{-4}$ ($3.19x10^{-4}$, $5.20x10^{-4}$)	$6.46x10^{-3}$ ($4.91x10^{-3}$, $8.01x10^{-3}$)	$7.89x10^{-2}$ ($6.00x10^{-2}$, $9.78x10^{-2}$)
Normal/non-sliced	$5.03x10^{-4}$ ($3.02x10^{-4}$, $7.71x10^{-4}$)	$7.72x10^{-3}$ ($4.63x10^{-3}$, $1.18x10^{-2}$)	$9.46x10^{-2}$ ($5.68x10^{-2}$, $1.45x10^{-1}$)
Hot-smoked fish			
ROP/sliced	$3.26x10^{-6}$ ($2.01x10^{-6}$, $5.02x10^{-6}$)	$3.72x10^{-5}$ ($2.29x10^{-5}$, $5.73x10^{-5}$)	$2.96x10^{-4}$ ($1.82x10^{-4}$, $4.55x10^{-4}$)
ROP/non-sliced	$1.51x10^{-6}$ ($7.53x10^{-7}$, $2.76x10^{-6}$)	$1.72x10^{-5}$ ($8.59x10^{-6}$, $3.15x10^{-5}$)	$1.37x10^{-4}$ ($6.83x10^{-5}$, $2.50x10^{-4}$)
Normal/sliced	$9.89x10^{-7}$ ($4.94x10^{-7}$, $1.81x10^{-6}$)	$1.36x10^{-5}$ ($6.78x10^{-6}$, $2.48x10^{-5}$)	$7.48x10^{-5}$ ($3.74x10^{-5}$, $1.37x10^{-4}$)
Normal/non-sliced	$2.14x10^{-6}$ ($1.32x10^{-6}$, $3.30x10^{-6}$)	$2.94x10^{-5}$ ($1.81x10^{-5}$, $4.52x10^{-5}$)	$1.62x10^{-4}$ ($9.97x10^{-5}$, $2.49x10^{-4}$)
Gravad fish			
ROP/sliced	$5.27x10^{-3}$ ($3.44x10^{-3}$, $7.33x10^{-3}$)	$5.86x10^{-2}$ ($3.82x10^{-2}$, $8.16x10^{-2}$)	$1.13x10^{0}$ ($7.35x10^{-1}$, $1.57x10^{0}$)
ROP/non-sliced	$4.58x10^{-4}$ ($2.16x10^{-3}$, $3.66x10^{-3}$)	$5.10x10^{-3}$ ($6.85x10^{-2}$, $4.08x10^{-2}$)	$9.80x10^{-2}$ ($5.08x10^{-1}$, $7.84x10^{-1}$)
Normal/sliced	$3.72x10^{-3}$ ($2.42x10^{-3}$, $5.17x10^{-3}$)	$3.73x10^{-2}$ ($2.44x10^{-2}$, $5.20x10^{-2}$)	$1.09x10^{0}$ ($7.09x10^{-1}$, $1.51x10^{0}$)
Normal/non-sliced	$3.23x10^{-4}$ ($2.30x10^{-3}$, $2.59x10^{-3}$)	$3.25x10^{-3}$ ($6.90x10^{-2}$, $2.60x10^{-2}$)	$9.46x10^{-2}$ ($5.10x10^{-1}$, $7.57x10^{-1}$)
Cooked meat			
ROP/sliced	$6.19x10^{-4}$ ($3.09x10^{-4}$, $9.28x10^{-4}$)	$1.48x10^{-2}$($7.41x10^{-3}$, $2.22x10^{-2}$)	$4.23x10^{-1}$ ($2.11x10^{-1}$, $6.34x10^{-1}$)
ROP/non-sliced	$6.25x10^{-4}$ ($3.79x10^{-4}$, $1.87x10^{-3}$)	$1.49x10^{-2}$ ($2.72x10^{-4}$, $4.47x10^{-2}$)	$4.19x10^{-1}$ ($1.59x10^{-2}$, $1.26x10^{0}$)
Normal/sliced	$6.29x10^{-4}$ ($3.14x10^{-4}$, $9.43x10^{-4}$)	$1.48x10^{-2}$ ($7.39x10^{-3}$, $2.22x10^{-2}$)	$4.17x10^{-1}$ ($2.09x10^{-1}$, $6.26x10^{-1}$)
Normal/non-sliced	$6.16x10^{-4}$ ($3.29x10^{-4}$, $1.85x10^{-3}$)	$1.48x10^{-2}$ ($2.80x10^{-4}$, $4.43x10^{-2}$)	$4.17x10^{-1}$ ($1.59x10^{-2}$, $1.25x10^{0}$)
Sausage			
ROP/sliced	$1.42x10^{-3}$ ($7.11x10^{-4}$, $2.84x10^{-3}$)	$1.62x10^{-2}$ ($8.12x10^{-3}$, $3.25x10^{-2}$)	$4.04x10^{-1}$ ($2.02x10^{-1}$, $8.17x10^{-1}$)
ROP/non-sliced	$7.25x10^{-4}$ ($1.96x10^{-5}$, $2.90x10^{-3}$)	$8.26x10^{-3}$ ($2.43x10^{-3}$, $3.30x10^{-2}$)	$2.07x10^{-1}$ ($8.51x10^{-3}$, $8.28x10^{-1}$)
Normal/sliced	$1.42x10^{-3}$ ($7.08x10^{-4}$, $2.83x10^{-3}$)	$1.61x10^{-2}$ ($8.04x10^{-3}$, $3.22x10^{-2}$)	$4.04x10^{-1}$ ($2.02x10^{-1}$, $8.08x10^{-1}$)
Normal/non-sliced	$7.14x10^{-4}$ ($1.96x10^{-5}$, $2.86x10^{-3}$)	$8.17x10^{-3}$ ($2.43x10^{-3}$, $3.27x10^{-2}$)	$2.04x10^{-1}$ ($8.51x10^{-3}$, $8.15x10^{-1}$)
Pâté			
ROP/sliced	$1.67x10^{-4}$ ($7.55x10^{-5}$, $4.42x10^{-4}$)	$1.15x10^{-3}$ ($5.19x10^{-4}$, $6.72x10^{-3}$)	$3.03x10^{-2}$ ($1.37x10^{-2}$, $1.33x10^{-1}$)
ROP/non-sliced	$2.20x10^{-3}$ ($2.14x10^{-5}$, $6.60x10^{-3}$)	$6.27x10^{-3}$ ($1.47x10^{-3}$, $1.64x10^{-2}$)	$6.54x10^{-1}$ ($3.88x10^{-2}$, $1.96x10^{0}$)
Normal/sliced	$4.45x10^{-3}$ ($1.78x10^{-3}$, $8.45x10^{-3}$)	$6.76x10^{-2}$ ($2.71x10^{-2}$, $1.29x10^{-1}$)	$1.32x10^{0}$ ($5.29x10^{-1}$, $2.51x10^{0}$)
Normal/non-sliced	$2.19x10^{-3}$ ($2.20x10^{-5}$, $6.58x10^{-3}$)	$3.20x10^{-2}$ ($1.47x10^{-3}$, $9.59x10^{-2}$)	$6.25x10^{-1}$ ($3.88x10^{-2}$, $1.95x10^{0}$)
Soft and semi-soft cheese			

Scenarios	Population subgroups		
	Healthy	Elderly	Pregnant
Sliced	2.04×10^{-5} (4.39×10^{-6}, 7.84×10^{-5})	1.15×10^{-4} (4.49×10^{-5}, 9.78×10^{-4})	1.98×10^{-3} (7.69×10^{-4}, 1.40×10^{-2})
Non-sliced	1.11×10^{-5} (5.27×10^{-6}, 2.01×10^{-5})	6.27×10^{-5} (2.98×10^{-5}, 1.14×10^{-4})	1.07×10^{-3} (5.10×10^{-4}, 1.95×10^{-2})

6851 ROP: reduced oxygen packaging. Numbers outside brackets represent 50th percentile; numbers between brackets represent 2.5 and 97.5th percentiles.

Appendix J – Uncertainty analysis of the *Listeria monocytogenes* generic quantitative microbiological risk assessment (gQMRA) model

6852 **Table 52:** Potential sources of uncertainty identified in the *Listeria monocytogenes* generic QMRA (gQMRA) model and qualitative assessment of the impact
6853 that these uncertainties could have on the final outcome

Component of assessment affected (e.g. subquestion, parameter, study, etc.)	Assumption/Data used	Brief description of sources of uncertainty	Direction of the effect on the number of cases[(a)]	Direction of the effect on the impact of factors on the number of cases[(a)]
Food categories	-Seven food categories (cold-smoked fish, hot-smoked fish, gravad fish, cooked meat, sausage, pâté, soft and semi-soft cheese) were assumed to represent RTE foods.	Other RTE food categories may contribute to human listeriosis	Both directions	Both directions
Prevalence	- A single value for prevalence of RTE food subcategory was assumed based on available occurrence data (BLS, and US data).	-Performance of detection methods and associated information bias, due to competition with background flora and poor recovery of *L. monocytogenes* on plates -Sampled products may not represent the food category	Both directions	Both directions
Initial concentration	-Initial concentrations (at decimal logarithm scale) were assumed to be distributed as a beta-general with a minimum equal to -1.69 and maximum equal to 6.1. The two other (shape) parameters of the beta-general distribution (a and β) are estimated based on data using a maximum likelihood estimation algorithm -BLS data were used for fish and USA data (Gombas et al., 2003) for meat and cheese distributions.	-Performance of detection methods and associated information bias, due to competition with background flora and poor recovery of *L. monocytogenes* on plates -Sampled products may not represent the food category -USA data may not represent EU	Both directions	Both directions
Time of storage	The remaining shelf life of a RTE food at the time of its purchasing was assumed to follow an exponential distribution. A variable named psl was used to introduce the variability in storage time which was described with a beta-pert distribution with a minimum, mode and maximum and mode equal to 0, 0.30 and 1.1 respectively. The storage time is derived by multiplying the psl by the remaining shelf life. -Storage time at consumer level (fraction of remaining shelf life) is considered independent of the food category;	-Storage time may differ between food categories --The used distribution for psl may not be appropriate -Sampled products may not represent the food category -psl description was based on expert knowledge since no data were available and may differ from reality	Both directions	Both directions

Component of assessment affected (e.g. subquestion, parameter, study, etc.)	Assumption/Data used	Brief description of sources of uncertainty	Direction of the effect on the number of cases[(a)]	Direction of the effect on the impact of factors on the number of cases[(a)]
Temperature of storage	-The temperature (T) of the consumer refrigerator was assumed normally distributed with a mean equal to 5.9°C and a standard deviation of 2.9°C based on literature data. -Storage temperature was considered independent of the food category. -Constant temperature was assumed during storage at consumer level (but variable between consumers) -Storage time and temperature were considered as independent factors.	-Performance of Temperature recording methods - The used distribution for T may not be appropriate -Storage temperature may differ among EU countries -Sampled refrigerators may not be representative -In reality temperature conditions are dynamic and not constant -Storage time and temperature are expected to be dependent factors due to spoilage	Both directions	Both directions
Growth	-The EGR at a specific temperature T is derived using this simplified secondary model, with T_{min} = −1.18°C -To describe the variability it was assumed that the EGR at 5°C is log-normally distributed. -The parameters of the Probability distributions of the EGR were estimated for the different food categories based on data from a review study carried out by Pérez-Rodríguez et al. (2017). -No lag time included (lag time was considered as completed from production to retail level). -No interaction (competition or metabiosis) with background flora was assumed. -A constant value for the maximum concentration was assumed.	- There may be some uncertainty of the used T_{min} value -The used distribution for EGR may not represent all sources of variability - Uncertainty of the used values of EGR distribution parameters - lag time may not be completed from production to retail -The background flora may affect the growth of the pathogen -The maximum concentration can vary depending on product, temperature, initial concentration and background flora	Both directions	Both directions
Consumption	-The average serving size (mass of RTE food ingested per meal) per category of food and per subpopulation as well as the TEO per year were estimated from the EFSA consumption data base. In total 14 subpopulations were considered (7 age groups for each male and female). -A single value (average) for both serving size and total number of eating occasions per year was used. Variability was not considered.	-Average serving size may not be the most representative parameter, for reflecting serving size -Uncertainty of data of EFSA consumption data base -Consumption data may vary among EU countries and over time -Variability in serving size and total number of eating occasions per year -Uncertainty around classification of food groups -General uncertainty with using 1–7 days diaries	Both directions	Both directions

Component of assessment affected (e.g. subquestion, parameter, study, etc.)	Assumption/Data used	Brief description of sources of uncertainty	Direction of the effect on the number of cases[(a)]	Direction of the effect on the impact of factors on the number of cases[(a)]
		to estimate overall consumption, e.g. no consumption during survey or high consumption		
Dose response	The mean of the log-Normal distribution of r for each of the 14 populations was estimated. With parsimony, the standard deviation was assumed to be the same for each subpopulation. The 14 means of r, were calculated based on the output of the exposure model, the average of the annual observed cases of listeriosis per subpopulation between 2008 and 2011 and the TEO per subpopulation.	-Uncertainty around using USA concentration data -Uncertainty around assumption of no variation of consumption within subpopulations -assumption of that variability between host factors and listeria factors are log-normally distributed -r values may vary among EU countries -DR data not independent from combined assessment; the same data as in exposure calibrated / anchored to the number of cases	Both directions	Both directions

BLS: EU-wide baseline survey; DR: dose response; EGR: exponential growth rate; psl: proportion of remaining shelf life; RTE: ready-to-eat; T: temperature; TEO: total number of eating occasions.
(a): Lack of data does not allow to estimate the direction of the uncertainty.