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Listeria monocytogenes risk assessment on cold smoked and salt-cured fishery products in Finland - A repeated exposure model

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ABSTRACT

Listeria monocytogenes causes severe consequences especially for persons belonging to risk groups. Finland is among the countries with highest number of listeriosis cases in the European Union. Although most reported cases appear to be sporadic and the maximum bacterial concentration of 100 cfu/g is not usually exceeded at retail, cold smoked and salt-cured fish products have been noted as those products with great risk especially for the elderly.

In order to investigate the listeriosis risk more carefully, an exposure assessment was developed, and laboratory results for cold smoked and salt-cured salmon products were exploited. *L. monocytogenes* exposure was modeled for consumers in two age groups, the elderly population as a risk group and the working-age population as a reference. Incidence was assessed by estimating bacterial growth in the food products at three temperatures. Bayesian estimation of the risk was based on bacterial occurrence and product consumption data and epidemiological population data.

The model builds on a two-state Markov chain describing repeated consumption on consecutive days. The cumulative exposure is probabilistically governed by the daily decreasing likelihood of continued consumption and the increasing bacterial concentrations due to growth. The population risk was then predicted with a Poisson distribution accounting for the daily probabilities of purchasing a contaminated product and the cumulative total probability of infection from its use.

According to the model presented in this article, elderly Finns are at a greater risk of acquiring listeriosis than healthy adults. The risk for the elderly does not fully diminish even if the products have been stored at the recommended temperature (between 0 and 3 °C). It can be concluded that the stage after retail, i.e. food handling and storage by consumer or professional kitchens, is essential to protection against listeriosis. The estimation model provides means for assessing the joint impacts of these effects.

1. Introduction

L. monocytogenes is a common foodborne bacterium capable of causing severe disease especially in persons with impaired immunity. It is found ubiquitously in the environment, including soil and wild animals (Weis and Seeliger, 1975). In addition, *L. monocytogenes* has a tendency to form biofilms on surfaces in food processing plants (Gudbjörnsdóttir et al., 2004). Therefore, *L. monocytogenes* is difficult to eradicate from foods. The bacterium is particularly resistant to many preservation methods, since it can grow in a wide range of pH values, at low water activity and under refrigerator temperatures (Ryser and Buchanan, 2013; Wareing et al., 2010). Almost all listeriosis cases are caused by *L. monocytogenes* contaminated foods whereas other transmission routes are rare (Mead et al., 1999). *L. monocytogenes* is generally found in foods that are eaten as such (i.e. ready-to-eat food), without additional heat treatment. Currently, *L. monocytogenes* concentration of < 100 cfu/g is accepted in products placed on the market during their shelf life in food capable of supporting the growth of the bacterium. According to Commission Regulation (EC) 2073/2005: "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." National *L. monocytogenes* surveys have been carried out to check compliance with this regulation.

In Finland, the reported incidence of listeriosis (1.22/100, 000 population) is higher than in many other European countries (0.47/100, 000 population) (EFSA, ECDC, 2017) and in the United States (0.26/

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100, 000 population) (Marder et al., 2017). In addition, the incidence has followed a slightly increasing trend in recent years, although this trend is not limited to Finland (Goulet et al., 2008). The reason for the higher incidence in Finland remains unsolved, although a similar trend has been observed in some other Nordic countries (EFSA, ECDC, 2017). One suggested reason for the higher incidence of listeriosis is the aging of the population, as 20% of the Finnish population has already reached the age of at least 65 years old, and the share of the elderly is increasing (Official Statistics of Finland, 2017). Cold smoked (CSS) and salt-cured salmon (SCS) are common delicacies in Finland and thought to account for the majority of listeriosis cases. The elderly eat them more often and their portions are bigger than those of the younger persons in Finland (EFSA, 2018). These products are also known to contain *L. monocytogenes* bacteria in their raw material, and often used vacuum or modified atmosphere packages (MAP) may also allow listeria growth in them while preventing other microbial growth. The Finnish Food Authority has given a national recommendation to store CSS/SCS at within 0 and 3 °C. (Finnish Food Authority, 2019) Retailers or manufacturers are obliged to store CSS/SCS between these temperatures according to Finnish national legislation (Ministry of Agriculture and Forestry statute 1367/2011). As no inactivation step is included in any processing state of these products, the possibility of *L. monocytogenes* contamination is high.

Although listeriosis is not as common as some other foodborne diseases, it has a higher hospitalization rate and case fatality rate (Gerner-Smidt et al., 2005; Ryser and Buchanan, 2013). In the European Union region listeriosis is assessed to be more severe than other foodborne diseases (EFSA, ECDC, 2017). This feature of listeriosis, together with the increasing incidence and sporadic appearance, makes it a serious threat to health. The need for risk assessment to produce the most effective control options is therefore notable.

In this study, *L. monocytogenes* risk assessment from retail to consumption was conducted for CSS and SCS to assess the risk within two population groups: the elderly (aged 65–74) and working-aged (aged 25–64, denoted as the reference population). Bayesian inferences were utilized as a modeling tool, as it features many advantages, including: utilizing the whole data set jointly as one, handling censored values and taking uncertainty into account (Albert et al., 2008, 2011; Busschaert et al., 2011; Greiner et al., 2013).

2. Materials and methods

2.1. Occurrence data and consumption data

L. monocytogenes concentration data were combined from three different *Listeria* surveys conducted in 2004–2010 (n = 1091). Details of the studies are presented in Table 1. Representative samples of CSS/SCS packages were collected from retail. The 2004 and 2008–2009 surveys were carried out at the national level under Finnish Food Agency's leadership. In the 2004 study, the samples were collected from the Finnish municipalities of Vantaa, Porvoo, Mikkeli and Kokkola and the surrounding municipalities. In the 2004 study, vacuum packed samples of CSS/SCS and samples from fish counters were collected. Samples from fish counters were found to be vacuum-packed and unpacked to the counter.

In the 2008–2009 survey, the samples were collected from the Helsinki metropolitan area and city of Turku. The place of sampling was considered irrelevant from national point of view, because there are only a few large-scale fish processing plants in Finland, and their products are delivered all over Finland. Only vacuum packed CSS/SCS were collected. The aim was to collect one sample from each sampled batch of CSS/SCS. The sample sizes (Table 1) were calculated based on the previous *Listeria* survey carried out in 2001, and in the 2008–2009 surveys the 2004 survey was exploited. The sample sizes were calculated on the assumption that the prevalence of *L. monocytogenes* was 14 ± 5% in CSS and 20 ± 5% in SCS. It was assumed that 95% of the CSS/SCS sold to consumers comes from large-scale producers and only 5% from local or small-scale producers. Thus, more samples from large-scale manufacturers were collected.

Table 1
Surveys used as a source of *L. monocytogenes* contamination data.

Survey year	n	Prevalence (%)	> 100 cfu/g (%)	Concentration range (cfu/g)
2004	596	15.7	2.3	< 1–250,000
2008–2009	453	31.8	1.8	< 10–5000
2010	42	21.4	2.4	< 10–360
Total	1091	22.8	2.1	< 1–250,000

The 2010 survey was a part of the European Union wide *Listeria* surveys. Details of the study design can be found in the Commission Decision 2010/678/EU. Briefly, a total of 272 samples of soft or semi-soft cheese, packaged smoked or fresh salted fish and packed heat-treated meat products were sampled from the cities of Helsinki, Espoo, Tampere, Vantaa, Turku, Oulu, Jyväskylä and Lahti. Only results for CSS/SCS were considered in this risk assessment, since the other product categories contained no positive samples.

In laboratory, the samples were analyzed qualitatively (presence/absence in 25 g) and quantitatively (concentration of *L. monocytogenes* in colony forming units per unit weight) with cultivation methods. ISO method 11290-1 (1996) was used for detection and ISO method 11290-2 (1998) for enumeration. Analyses were carried out at the use-by date in surveys carried out at 2004 and 2010, or five days prior to the use-by date in the 2008–2009 survey, or somewhere between these, as analysis was carried out before the use-by date if it was not workday. The manufacturing date was only recorded for 31 samples. For these samples, the selling time ranged from 2 to 30 days. Some of the samples (N = 8) had to be rejected due to insufficient documentation and enumeration had not been done for 21 samples that were found positive in the qualitative analysis. For the modeling, the positive samples were assumed to have minimum concentration of 1/25 = 0.04 cfu/g and the limit of quantification was 1, 10, 40 or 100 cfu/g, which was set as the upper limit of concentration for samples below the quantification limit.

In the exposure assessment, two populations were considered. The working-age population (aged 25–64 years) was about 2.8 million persons (about 50% of the whole Finnish population). The elderly population (aged 65–74 years) was 650, 000 persons (about 10% of the Finnish population) (Official Statistics of Finland, 2017). These two populations were chosen based on the available food consumption data.

For the two populations, food consumption data was acquired from the The National FINDIET 2007 survey. FINDIET surveys are national food consumption studies repeated every five years by the National Institute for Health and Welfare. The purpose of these studies is to examine the food consumption and nutrient intake of the Finnish adult population. The study used the 48-hour recall method to interview a representative sample of 2038 adults aged 25–74 years (Paturi et al., 2008; Pietinen et al., 2010). The data used in this risk assessment was collected between January and March 2007 (Pietinen et al., 2010) and these were divided into two populations: the elderly (age 65–74 years, n = 463) and the reference population (25–64 years, n = 1575). No data were available for special groups, such as infants or pregnant women. A total of 100 persons had eaten CSS/SCS on at least one of the survey days (63 on day one and 61 on day two). There were 29 CSS/SCS users in the elderly population and 71 in the reference group. For simplicity, one portion was considered as all the CSS and SCS consumed during one consumption day. Only CSS/SCS eaten as such or in uncooked dishes were included. Two types of information were obtained from this data: daily consumption (g/day) and consumption frequency on the population level.

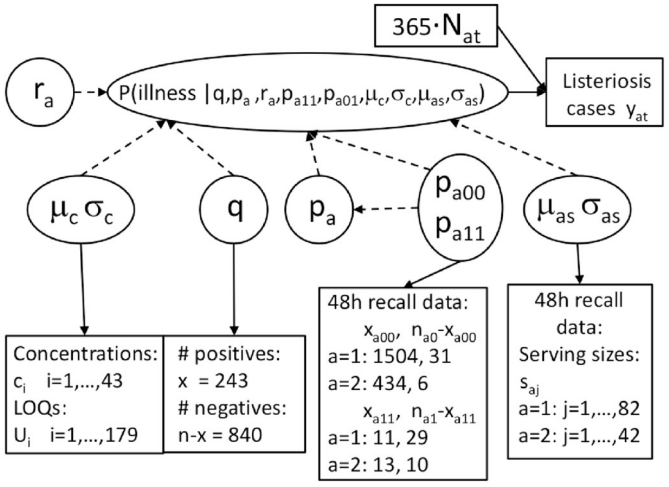

Fig. 1. Directed acyclic graph (DAG) of the model parameters (ellipses) and data (rectangles). Solid arrows correspond to distributions, dashed arrows to deterministic functions. Age-group denoted by subindex *a*, year by *t*. See equations below for full functional description.

2.2. Model

Computations of the risk assessment model were carried out using R software with the R2OpenBUGS package (Sturtz et al., 2005) and OpenBUGS software (Lunn et al., 2013). R-scripts were developed for reading and processing of the data files, and then formatting of the inputs suitable for BUGS-model syntax in which the actual model was defined. OpenBUGS then returns the MCMC output back to R, where the results are processed and graphics are produced. The full Bayesian model consists of three linked modules: a model for the occurrence data, a model for the consumption data and a predictive model for the total number of cases in the population. Finally, the model can also use the reported number of cases to 'calibrate' the dose–response function for the target population, hence combining bottom-up and top-down approaches for any specified age-groups, Fig. 1. The novelty is that it enables all unknown parameters to be estimated jointly from the explicitly defined data set, in a single compact model, without a separate disconnected treatment for each parameter. As a result, the uncertainty distribution of parameters is truly multidimensional and reflects the information contained in that data.

In the consumption frequency model, the probability (for any day, any consumer in the age-group) of consuming CSS/SCS was constructed. Since the consumption data consisted of food recalls for two consecutive days, they are informative about transition probabilities between consumption ("1") and no-consumption ("0") over consecutive days. For most consumers in the data (80%), only one serving per day was eaten, and we simply assume one or zero servings of CSS/SCS per day in the modeling. The counted number of transitions ($0 \to 0$, $0 \to 1$, $1 \to 0$, $1 \to 1$) in the age-group provide necessary information for the corresponding group specific transition probabilities (p_{a00}, p_{a01}, p_{a10}, p_{a11}) between two days, for group *a*. Interpreting daily consumption events in this way as a two-state Markov chain, the long-term equilibrium probability for consumption can be written as an expression of the transition probabilities: $p_a = (1 - p_{a00})/(1 + p_{a10} - p_{a00})$. The expression follows from basic theory of Markov chains (Karlin and Taylor, 1975). Hence, this was taken as the generic consumption probability (p_a) for an unspecified day. The probability to start consumption is thus $(1 - p_a)p_{a01}$. This is the simplest model that captures the consumption pattern where the probability to consume depends on the consumption event (yes/no) of the previous day. Food diary data over several days could be similarly utilized, which also would allow more refined modeling of dependency structures over days. The consumption amount model consists of a log-normal distribution for the reported daily consumption amounts (servings, g/day), if consuming CSS/SCS.

In the occurrence model, the probability of contamination (q) of CSS/SCS was defined as a binomial parameter based on the sampled CSS/SCS and the number of positive samples. The concentration model consists of a log-normal distribution for the measured bacterial levels (cfu) in the positive samples, also accounting for values below the level of enumeration as interval-censored data. No false negatives were assumed since they would necessarily be very low concentrations which would not have large effect on the results, and a zero-inflated model would increase computational burden.

Once exposed to contaminated CSS/SCS, the probability of illness (i.e. an infection triggering the incubation leading to illness) in age-group a was evaluated as in the exponential dose-response model $1 - \exp(-r_a D)$ where D is the expected population mean dose for some consumption. (The actual unknown dose is Poisson distributed from serving to serving with mean D, either because of uniformly random distribution of bacteria in the product or because of effective mixing of it into servings. The Poisson distribution combined with single hit model results to the exponential model which is common in the literature (Schmidt et al., 2013, Teunis et al., 1999). This assumption is generally used even though for example Cortesi et al. (1997) and Lappi et al. (2004) have found evidence that *L. monocytogenes* may be unevenly distributed in CSS/SCS (Cortesi et al., 1997; Lappi et al., 2004).) The parameter r_a for the age group could be either drawn from the literature or, as explained below, treated similarly with other unknown parameters in the model, based on the full data set. The mean dose on the first consumption day of a randomly purchased CSS/SCS is $D_{a1}{}^* = \exp(c^* s_a{}^*)$ where c^* is the predicted log-concentration and $s_a{}^*$ the predicted log-consumption amount, according to distributions N $(\mu_c, \sigma_c{}^2)$ and N$(\mu_{as}, \sigma_{as}{}^2)$, respectively. Subindex c denotes 'concentration', and as denotes 'serving' in age group a. Thus, the population illness probability for a random consumer, on 1st day, $P_1(\text{ill} | r_a, \mu_c, \sigma_c, \mu_{as}, \sigma_{as})$, given the parameters, is the expected value

$$E_D(P_1(\text{ill} \mid r_a, D_{a1}^*)) = \int_0^\infty P_1(\text{ill} \mid r_a, D_{a1}^*) P(D_{a1}^* \mid \mu_c, \sigma_c, \mu_{as}, \sigma_{as}) dD_{a1}^* \quad (1)$$

There is no closed form solution for the integral, but it can be Monte Carlo approximated by evaluating the mean of many CSS/SCS specific simulated probabilities. That is, generating K mean doses $\exp(c^* s_a{}^*)$ from K values of c^*, $s_a{}^*$ each generated from $c^* \sim N(\mu_c, \sigma_c{}^2)$ and $s_a{}^* \sim N(\mu_{as}, \sigma_{as}{}^2)$, and evaluating the average of the resulting K probabilities $P_1(\text{ill} | r_a, D_{a1}{}^*)$. For Bayesian inference of the core model parameters of interest, the Bayes theorem for the full model with epidemiological data requires evaluating population illness probabilities for any given set of core parameters r_a, μ_c, σ_c, μ_{as}, σ_{as}. In the absence of exact expression for the integral in Eq. 1, the simulated approximation (based on 100 replicate CSS/SCS) was a substitute at every iteration of MCMC, i.e. $\widehat{P}_1(\text{ill} \mid r_a, \mu_c, \sigma_c, \mu_{as}, \sigma_{as})$. This increases computational burden, though. Similar problems are encountered with e.g. ecological models with approximated detection probabilities (Bonner and Schofield, 2014). Furthermore, the illness probability needs to be likewise evaluated for all consecutive days $d = 1, \ldots, 7$, with concentration value taken from the growth model each day (starting day one with different c^* for each replicate CSS/SCS) and random serving size per day.

The overall population probability of illness is then the product of the necessary probabilities: P(consumption started), P(contamination), and P(illness, given mean dose) over a possible consumption episode. If the consumption day is the same as the date of purchase, the concentration distribution logN$(\mu_c, \sigma_c{}^2)$ at retail applies for predictions with a mean of μ_c. The probability p_{a11} of consuming CSS/SCS again the next day was fairly high (from food diary counts: ≈57% (13/23) for the elderly, ≈28% (11/40) for the reference population). Most (80%) CSS/SCS, was consumed in private homes, whereas only about 10% was eaten in a restaurant. This may indicate that it is common to purchase a sufficiently large amount of CSS/SCS and continue its consumption over several days. The probability of switching to another CSS/SCS while the

first purchased CSS/SCS is unfinished was considered negligible. Since prolonged storage of the product is important for the risk of bacteria growth, for simplicity we assume a Poisson process for purchasing where $(1 - p_a)p_{a01}$ is taken as the probability of consumption on the day of purchase (age-group a), and we model the consequent consumption days based on the transition probabilities. The total probability of illness accounts for the possibility of serial exposures until the first no-consumption day occurs or a week is finished.

$$P(\text{ill} \mid q, p_a, r_a, p_{a11}, \mu_c, \sigma_c, \mu_{as}, \sigma_{as}) = (1 - p_a)p_{a01}q\Bigg(P_1(\text{ill} \mid r_a, \mu_1, \sigma_c, \mu_{as}, \sigma_{as}) + \sum_{d=2}^{7}\prod_{i=1}^{d-1}\Bigg(1 - P_i\Big(\text{ill} \mid r_a, \mu_i, \sigma_c, \mu_{as}, \sigma_{as}\Big)\Big)p_{a11}^{d-1} P_d(\text{ill} \mid r_a, \mu_d, \sigma_c, \mu_{as}, \sigma_{as})\Bigg) \quad (2)$$

This probability hence depends on the eight core parameters, also shown in the DAG of Fig. 1. Note that the model is a single-hit model for discrete time steps representing consumption events and the exposure period (here a week) which can lead to only one illness per one individual. If the infection (leading to illness) is triggered according to dose-response probability for some day, then the outcome will be illness regardless of possible additional exposures during the following days. Therefore, it is sufficient to write the probability of illness as the probability of first infection (leading to illness) on day d, summed over days. Consequently, the probability of first illness from the exposure on the ith day requires avoiding infection during the previous days while still continuing consumption. This can be extended to an arbitrary time, although the probability of continued consumption of the same product for six consecutive days ($p_{a11}{}^6$) is already very small. However, for the risk this could be compensated by rapid bacteria growth. Given that the consumption of contaminated CSS/SCS does occur on day d, the illness probability P_d(ill) depends on the day-specific level of contamination (μ_d for day d) which was determined from the growth model function $\mu_d = f(\mu_{d-1})$ with an initial level $\mu_1 = c^*$ and an assumed constant temperature in a home refrigerator. The default was 7 °C and scenarios with 3 and 10 °C (Finnish Food Authority, 2019; Marklinder et al., 2004). Modeling of the variability of temperatures was not possible because there were no such data.

Exposure to listeria from CSS/SCS is a relatively rare event. However, once it occurs, exposure may continue repeatedly over some days. The distribution of the annual total number of disease cases was approximately modeled as a Poisson distribution with parameter $\lambda_a = N_a 365 P(\text{illness} \mid q, p_a, r_a, p_{a11}, \mu_c, \sigma_c, \mu_{as}, \sigma_{as})$. This is based on the population size N_a and the probability of illness triggered on any day of the consumption period, and assuming all cases are due to CSS/SCS. In general, more food types could be added if data become available, or a source attribution fraction applied based on extended modeling.

Model parameters were estimated by computing (in OpenBUGS) the posterior distribution defined by the full likelihood and the priors:

$$\begin{aligned} P(q, p_a, r_a, p_{a11}, p_{a00}, \mu_c, \sigma_c, \mu_{as}, \sigma_{as} \mid \text{occurrence data, consumption data, reported cases}) \propto\ & \text{Bin}(x \mid n, q) \\ & \times \textstyle\prod_{i=1}^{43} N(c_i \mid \mu_c, \sigma_c^2) \\ & \times \textstyle\prod_{i=1}^{179} [F(U_i \mid \mu_c, \sigma_c^2) - F(\log(1/24) \mid \mu_c, \sigma_c^2)] \\ & \times \Big[\textstyle\prod_{a=1}^{2}\prod_{j=1}^{J_a} N(s_{aj} \mid \mu_{as}, \sigma_{as}^2) \\ & \times \text{Bin}(x_{a00} \mid n_{a0}, p_{a00}) \\ & \times \text{Bin}(x_{a11} \mid n_{a1}, p_{a11}) \\ & \times \textstyle\prod_{t=2004}^{2010} \text{Poisson}(y_{at} \mid N_{at} 365 P(\text{ill} \mid q, p_a, p_{a11}, r_a, \mu_c, \sigma_c, \mu_{as}, \sigma_{as})) \\ & \times P(p_{a00}, p_{a11}, \mu_{as}, \sigma_{as}, r_a)] P(q, \mu_c, \sigma_c) \end{aligned} \quad (3)$$

The full likelihood function is the product of the conditional probabilities for the number of positive samples (x/n), exact log-concentrations (c_i), log-concentrations below the log-limit (U_i), log-portion sizes in age groups (s_{aj}; $j = 1, \ldots, J_a$; $J_1 = 82$, $J_2 = 42$), the number of no-consumption days after a no-consumption day (x_{a00}/n_{a0}) and the number of consumption days after a consumption day (x_{a11}/n_{a1}) in age-groups, and finally the observed reported cases of illness (y_{at}) during the years 2004–2010 in age groups. Prior distributions for q, p_{a00}, p_{a11} were uniform U(0,1), prior for logit(r_a) was N(0,1000), priors for μ_c, μ_{as} were N(0,1000), and priors for σ_c, σ_{as} were uniform over a wide range, U(0,100).

The growth of *L. monocytogenes* during home refrigerator storage was modeled using a logistic growth model (4) from Mejlholm and Dalgaard (2015) with cardinal parameter secondary growth model (5) from Mejlholm et al. (2014); Mejlholm and Dalgaard (2007, 2009, 2015). The model takes into account several environmental factors, of which four were considered in this study: temperature, salt content, pH and phenolic compounds from smoking. The value of a_w was estimated from the salt content according to Eqs. 7 and 6, (Ross and Dalgaard, 2004). The temperatures used in growth model 5 were 3 °C (the recommended storage temperature) (Finnish Food Authority, 2019), 7 °C (the average temperature in a consumer refrigerator) (Kennedy et al., 2005; Marklinder et al., 2004) and 10 °C (the worst-case scenario) (Marklinder et al., 2004). It was not possible to know the precise starting time for bacterial growth. Hence, the measurements at retail were taken to represent the initial concentration levels for the consumer, with no lag time. The growth model was applied to calculating the predicted mean bacterial concentration during repeated consumption days.

$$\log N_t = \log\left(\frac{N_{\max}}{\left(1 + \left(\frac{N_{\max}}{N_0} - 1\right)\right)\exp(-\mu_{\max} t)}\right) \quad (4)$$

$$\mu_{\max} = 0.419\left(\frac{T + 2.83}{25 + 2.83}\right)^2 \frac{a_w - 0.923}{1 - 0.923}(1 - 10^{4.97 - pH})\frac{32.0 - P}{32.0} \quad (5)$$

$$WPS(\%) = \frac{C_{\text{NaCl}}(\%)100}{100 - \text{dry matter}(\%) + C_{\text{NaCl}}} \quad (6)$$

$$a_w = 1 - 0.0052471 WPS(\%) - 0.00012206 WPS(\%)^2 \quad (7)$$

where N_t is the *L. monocytogenes* concentration at a time t, N_0 is the initial concentration, N_{max}, the maximum population density ($10^{8.5}$), μ_{max} the maximum specific growth rate of *L. monocytogenes*, T, a_w, pH (6.14), P (concentration of phenolic compounds, 5 ppm) and dry matter (30%) are product characteristics of CSS/SCS, and WPS(%) is the water-phase salt. The salt content (3.17%) was calculated from the Fineli database (Reinivuo et al., 2010) and concentration of phenolic compounds was obtained from (Mejlholm and Dalgaard, 2015).

The reported number of cases from years 2004–2010 was used as data for the model, thus calibrating the parameters to their likely values in the studied population. For elderly population, there were 11, 11, 13, 10, 8, 10 and 21, and for the reference population, there were 11, 10, 15, 12, 11, 8 and 17 annual cases (THL, 2018). Effectively, the 'response' in the dose–response model then becomes re-interpreted as the reported number of cases, and it is assumed that all these cases result from the consumption of CSS/SCS, because the attribution of other sources was not modeled. To demonstrate the effect of dose–response model parameter r to the predicted annual listeriosis cases, dose–response obtained in this study was compared to models from Lindqvist and Westöö (2000), FAO and WHO (2004), and Pouillot et al. (2015). These were all exponential dose–response models that, similarly to the model described in this study, separately considered the non-susceptible and susceptible groups (or only the susceptible group in the study of Lindqvist and Westöö (2000)). Since the populations are not exactly the same, this underlines the need for new estimates with data from the specific target population.

3. Results

The posterior distribution was simulated by MCMC in OpenBUGS with 10,000 iterations including 1000 burn-in iterations, consumption amounts for each day, all predicted per each MCMC iteration. Total iterations thus amount to 6.3 million serving sizes, 0.9 million initial concentration values, and 9000 values for each underlying core model parameter. The predicted initial *L. monocytogenes* concentration was moderately low (mean 97 cfu/g, median 3 cfu/g). *L. monocytogenes* contaminated CSS/SCS packages tend to have a low bacterial concentration, but on a few rare occasions the concentration is very high (thousands of cfu/g). About 80% of the positive samples were below the quantification level. Only a small proportion, i.e. about 10%, of the predicted (non-zero) concentrations exceeded the 100 cfu/g limit in contaminated CSS/SCS packages, while the prevalence was estimated to be on average 0.22 (posterior median) 95% CI [0.20,0.25].

CSS/SCS are not everyday food: the proportion of CSS/SCS consumption days was estimated as 3.2% in the elderly population and 2.8% in the reference population (posterior medians). On average, for an elderly or reference individual, this means 12 or 10 consumption days per year, respectively. However, if CSS/SCS is eaten, the probability of consumption on the next day is relatively high: about 55% in the elderly population and 27% in the reference population (posterior medians). Consumed amounts (only days when consumed) were on average 85 g in the elderly population and 71 g in the reference population (posterior medians for mean serving size). Means calculated from the data were 86 g and 70 g, respectively. All CSS/SCS was assumed to be eaten as such without any heat treatment.

The effect of storage temperature and time on the risk of illness is illustrated in Fig. 2 for the elderly population and for the working-aged population. While the incidence of listeriosis per 100,000 persons for the elderly population in the reference storage scenario of 7 °C was estimated 3.1, (median, CI 95% [0.4,28.6]), in the 3 °C storage scenario the incidence decreased to 0.9, (median, CI 95% [0.1,8.1]), and in the 10 °C storage scenario it increased to 16.1 (median, CI 95% [1.9127.2]). In the working-age population, the incidence per 100,000 persons was estimated 0.5 (median, CI 95% [0.1,4.9]), for the reference storage temperature of 7 °C, and the scenarios predicted 0.3 (median, CI 95% [0.0,2.7]), for 3 °C storage and 1.0 (median, CI 95% [0.1,9.0]), for 10 °C storage.

The *r*-parameter of the exponential dose–response model was estimated based on full data including occurrence data, consumption data and reported cases of illness from 2004 to 2010 with the population sizes of the two age groups for these years. The absolute number of annual cases was very similar for both the elderly and reference populations. However, since the population size of the elderly was over four times smaller than reference population size, the person risk of illness must be similarly larger. The risk of illness depends not only on the *r*-parameter of the dose–response relationship, but also on consumption amounts, the frequency of consumption and the tendency to keep consuming (the same) food over consecutive days when bacterial growth can occur. All the consumer dependent parameters r, μ_s, σ_s, p, p_{11}, p_{01} together contribute to the resulting disease counts. Since they were estimated together, their marginal 2D-distributions reveal the joint uncertainty and plausible parameter combinations, Fig. 3. A clear difference in the r parameter for the elderly 95%CI $[6.2 \times 10^{-11}, 4.7 \times 10^{-9}]$ and the reference 95%CI $[3.9 \times 10^{-11}, 1.4 \times 10^{-9}]$ could not be concluded as the CIs were largely overlapping. There is difference in the consumption parameters that could clearly increase the cumulative overall risk of the elderly, if they tend to consume the same ready-to-eat CSS/SCS over several days, together with risky storage temperatures and larger servings. Therefore, estimation of one parameter from this actual population data cannot be separated from the estimation of other parameters since they all contribute to the observable outcome. If a contaminated CSS/SCS was bought, the overall risk over the following days could either increase or decrease depending on which of the competing effects more rapidly overrides the other: bacterial growth or the probability of stopping consumption. Both are described by model parameters.

The effect of the exponential dose–response parameter *r* was studied by applying different point estimates for *r*-parameter values to this model from various literature sources and predicting the incidence without using data on reported cases, i.e. only bottom-up predictions. A point estimate is difficult to choose from literature because of known and unknown differences between populations that are not easily adjusted or quantified without a similar data base. Orders of magnitude differences in assumed values of *r* can affect the results quite much (see Table 2). Therefore, the full model provides a data based method specific for the target population.

4. Discussion

As the number of listeriosis cases continues to increase in Finland, the need for effective risk management options becomes evident. This study described the use of a novel risk assessment model to evaluate the incidence of listeriosis caused by the consumption of cold-smoked and salt-cured salmon (CSS/SCS) and the effect of storage temperature on it. The results of this risk assessment showed that those preparing and handling food may play a critical role in the incidence of listeriosis. In the recommended storage scenario (3 °C), on average only five elderly persons caught listeriosis. If the storage temperature was set to 10 °C, about 80 elderly persons could acquire listeriosis. In the reference group, the respective number of cases was 9 and 28.

The current model takes into account the possibility of continuing consumption of the same (contaminated) package of CSS/SCS, rather than assuming independent consumption days. The data used in this model support this assumption, as the probability of consumption on a particular day is relatively high when CSS/SCS has been consumed on the previous day. This suggests that the same package may be consumed over multiple days, even though there is no certainty, since the data reports only consumption. Further support for this assumption is given by the fact that CSS/SCS is most often eaten in private homes (about 80% of reported consumption) and only about 10% is eaten in restaurants or cafés. It is also possible that some of the SCS eaten at home is self-made, thus leading to higher consumption in private homes compared to restaurants or cafés.

The probability of illness for the elderly population increases over consecutive days of consumption, and becomes higher than in the reference population, due to higher probability of continued consumption and larger serving sizes. It is clear that the storage temperature and time then become critical.

The results for a broad age group may not represent healthy individuals, since the reported cases might have belonged to high-risk groups within the age group. A more informative stratification of population data would be needed to run the model for meaningful and more homogeneous population subgroups rather than broad age groups. In addition, the elderly population over 75 years was left out from this risk assessment, as there were no data on them. Here, we also made a simplified assumption that consumption within the studied groups is similar; however, other factors than age (such as place of residence, gender and income) may also affect the consumption patterns. The population used for the modeling was the entire (stratified) population of the country, and the estimates are thus adjusted top-down (with reported cases) and bottom-up (with occurrence and consumption data), rather than only involving bottom-up extrapolation from predictive models. Also, the method is based on actually observed data, eliminating the need to separately assess the r-parameter, as in the approach used by Lindqvist and Westöö (2000) and FAO and WHO (2004), among others. With better knowledge of the number of cases in different susceptible groups and occurrence data from other relevant food groups, this model would probably yield more nuanced results.

The dose–response model can have a major impact on the predicted

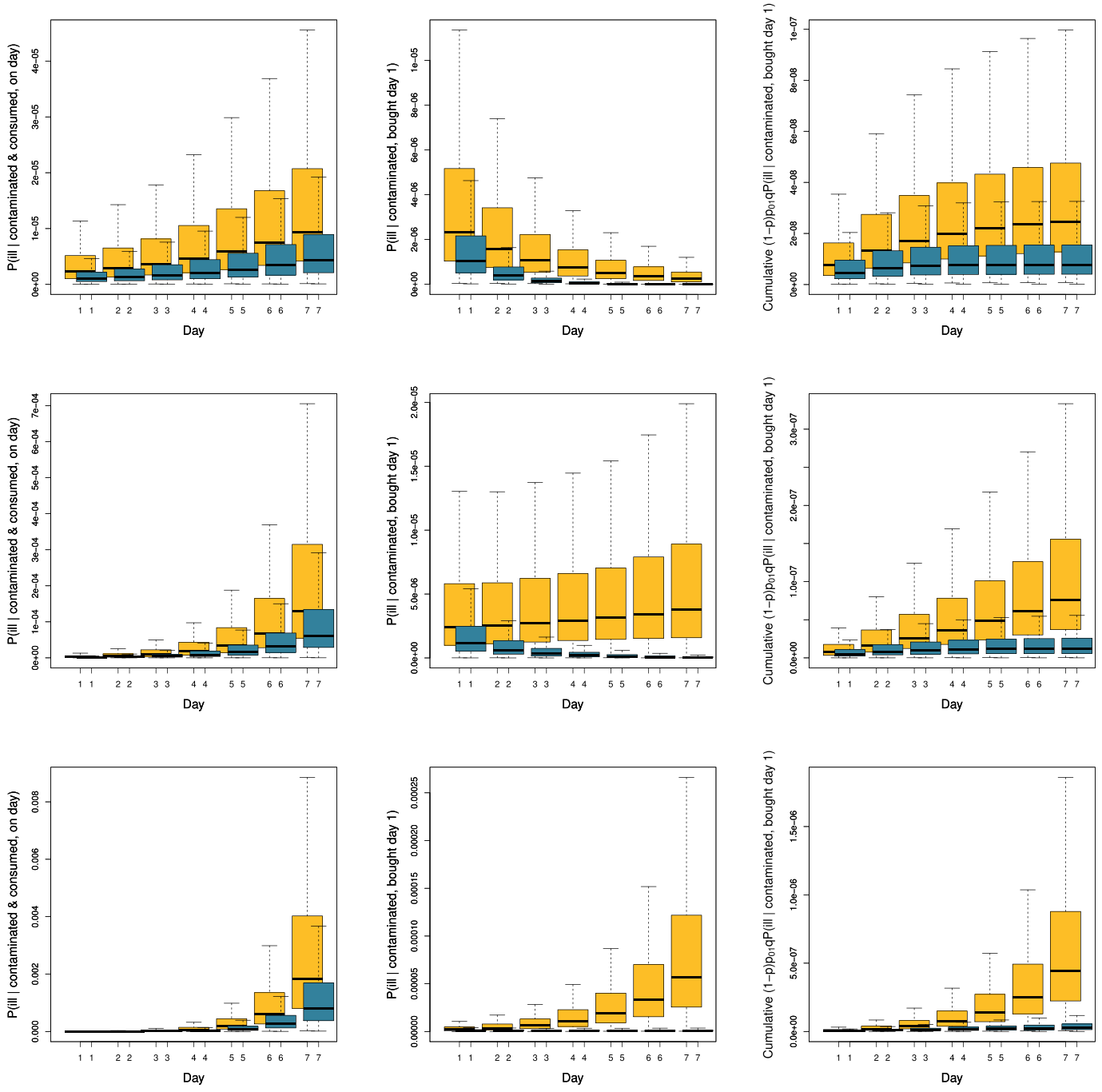

Fig. 2. Top row: temperature 3 °C. Middle row: temperature 7 °C. Bottom row: temperature 10 °C. Left column: probability of illness if contaminated CSS/SCS is eaten. Middle column: probability of illness if contaminated CSS/SCS is bought on the 1st day. Right column: overall cumulative probability of illness. Elderly ■ and reference ■.

incidence (Table 2) in predictive modeling with a bottom-up approach. The parameter *r* in an exponential dose response model is commonly interpreted as the probability of one (*L. monocytogenes*) cell causing illness (or infection). The value is often assumed to be constant for a given host population. From the models examined here, those by Lindqvist and Westöö (2000) and FAO and WHO (2004) used a single value (i.e. point estimate) for this parameter. The purposefully conservative model by Lindqvist and Westöö (2000) predicted the highest incidence compared to the other models, as could be expected. The FAO and WHO (2004) risk assessment used a similar approach to Lindqvist and Westöö (2000) with a different data set. Their dose–response model gave a substantially lower estimate of the incidence. Compared to the observed incidence, this model may be even too optimistic. The model presented by Pouillot et al. (2015) also took into account the variability of infectivity among different *L. monocytogenes* strains and host susceptibilities, making this approach more realistic. However, none of these predictive models led to case counts similar to the observed counts. In predicting observable incidence records in different populations, single point estimates of *r* are not universally applicable. In our approach, the *r*-parameter is jointly estimated with the rest of the parameters. Although Pouillot et al. (2015) describes variability of *r*, it does not provide a closed form solution of the dose-response probability and therefore it does not easily lend itself for parameter inference for the full set of parameters in Bayesian models with MCMC, or even

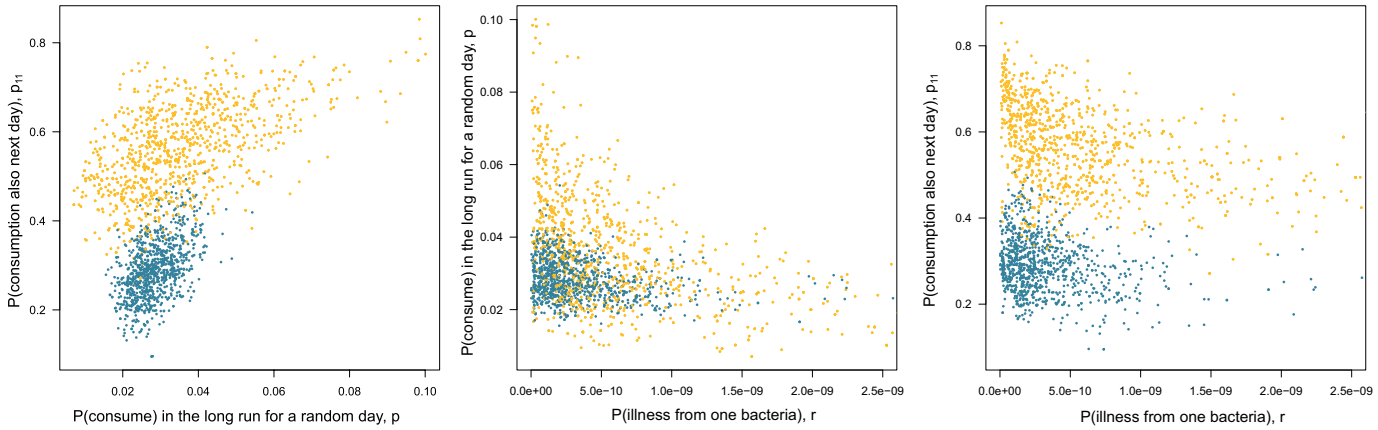

Fig. 3. Comparison of 2D-posterior distributions for (p,p_{11}), (r,p), (r,p_{11}). Elderly • and reference •.

predictions with fixed underlying variability parameters. Moreover, variability estimation should be conducted with respect to the available data that is population subgroup specific. A possible next step for inference could be to apply approximations as with MC within MCMC.

One recommended way to 'validate' a microbiological risk assessment model is to compare the results with actually observed illness cases. During the last 22 years, the annual incidence of listeriosis per 100,000 persons has ranged from 0.25 to 0.79 in the age group 25–64 years and 0.92 to 4.79 per 100,000 persons in the age group 65–74 years (THL, 2018). Currently, no reliable estimation of listeriosis underreporting exists, although, for example, Mead et al. (1999) have presented some evaluations. However, as listeriosis is more severe than many other foodborne illnesses and this risk assessment only considered the more severe invasive listeriosis and not the gastroenteritis form of the disease, it is likely that underreporting is not as significant a source of error as in many other foodborne diseases. The severe symptoms of invasive listeriosis more often lead to hospitalization than gastroenteritis and thus a larger proportion of cases are observed (Mead et al., 1999). Using the most realistic temperature scenario of 7 °C based on current knowledge, the model-predicted incidence per 100,000 was 1.2 (median) 95%CI [0.1,4.9] for the reference group and 3.1 (median) 95%CI [0.4,28.6] for the elderly, as a result from the combined uncertainty of model parameters. The medians fall between observed ranges, but intervals have higher upper bounds as might be expected for prediction uncertainty. However, this risk assessment assumed that only one vehicle for listeriosis is linked to the case count. The 10 °C storage temperature scenario increased the predicted cases clearly above the observed numbers, but was somewhat unrealistic. Consumer refrigerator temperature studies have revealed that only a minority (4–11%) of consumers have a temperature this high or higher (Marklinder et al., 2004). However, this scenario is still a good indication of the effect of consumer behavior. Although refrigerator temperatures do vary, the scenario results with a constant temperature are easy to interpret, and data on the temperature distribution in Finland were not available.

Limited knowledge causes limitations for the model. There are currently no reliable estimates of the dose–response relationship for *L. monocytogenes* in humans. Due to high mortality in listeriosis, volunteer challenge trials cannot (and should not) be performed. To overcome this limitation, the response in this study was interpreted to describe illness cases severe enough to require medical attention. Thus, this model probably ignores the gastroenteritis form of listeriosis almost completely, as such cases are usually mild and unlikely to require medical attention (Vázquez-Boland et al., 2001). Secondly, it is likely that some strains of *L. monocytogenes* are more virulent than others (Nexmann Larsen et al., 2002) but knowledge of these differences is limited and it was therefore assumed that all the strains are equally capable of causing listeriosis. Thirdly, the Finnish *L. monocytogenes* surveillance data used in this risk assessment were analyzed at the end of their shelf-life. In risk assessment, data on concentrations on consumption days would rather be needed. This leads to a need to make assumptions whose validity cannot be fully verified. In addition, the surveys studied more large-scale producers than small-scale producers. If the small-scale producers would have a larger *L. monocytogenes* occurrence, it would lead to a higher risk in persons using mostly products from small-scale producers. Lastly, data on consumer behavior are still scarce (Redmont and Griffith, 2003). Our model lacks some ways of storing or handling CSS/SCS (such as freezing or heating) or eating from two different packages. If data were available on the amounts purchased, this could be accounted for as a limitation on the number of servings (days) until the product has been entirely consumed. Currently, the model only assumes day-by-day probabilities for continuing consumption over some theoretical maximum number of days, which was cut off at one week.

CSS/SCS is not the only risk food group for *L. monocytogenes*. Other risk assessment have evaluated food groups such as deli meats, soft

Table 2
Predictions with different dose–response parameters r. * The model by Pouillot et al. (2015) considered healthy individuals **median.

		Incidence				
Model	**r**	**Mean**	**Sd**	**2.5th %ile**	**median**	**97.5th %ile**
Elderly						
Lindqvist and Westöö (2000)	5.6×10^{-10}	102	130	8.2	61.2	442.9
FAO and WHO (2004)	5.85×10^{-12}	1.1	1.4	0.1	0.6	4.7
Pouillot et al. (2015)*	1.47×10^{-13}**	0.03	0.04	0.00	0.02	0.1
Current model	4.2×10^{-10}**	6.4	20.4	0.4	3.1	28.6
Reference						
FAO and WHO (2004)	5.34×10^{-14}	0.00	0.00	0.00	0.00	0.00
Pouillot et al. (2015)*	7.82×10^{-15}**	0.00	0.00	0.00	0.00	0.00
Current model	2.6×10^{-10}**	1.2	6.9	0.06	0.5	4.9

cheeses, frozen vegetables, dairy products and ready meals as posing a risk for listeriosis (FDA and FSIS, 2003; Little et al., 2010). However, in Finland these product groups have been studied less. Therefore, more information would be needed in order to carry out a larger risk assessment taking into account multiple infection sources. With only one food type, the model assumes that all cases are due to this food type. Also, underreporting of the cases was not accounted for, but could be added, provided with some data on reporting. In addition, only limited knowledge exists on *L. monocytogenes* virulence and the dose–response relationship, which causes uncertainty in the model.

This risk assessment revealed that although CSS/SCS products mainly comply well with the European regulations, consumer behavior may substantially affect the listeriosis risk. By following the national recommendations for fish storage (Finnish Food Authority, 2019), the incidence of listeriosis caused by the consumption of CSS/SCS would be almost negligible. Limitation of the shelf life of CSS/SCS could be an effective control measure, but not all consumers comply with use-by-dates (Marklinder et al., 2004). Moreover, it would also probably lead to larger amounts of waste. In Finland, considerable effort has been put into improving the *L. monocytogenes* contamination situation in processing and retail steps (Nakari et al., 2014). However, affecting the behavior of consumers is more difficult. Because of malpractices in the storage habits of CSS/SCS, controlling the concentrations alone is not adequate, and surveillance should also target the lowering of the prevalence. Even small concentrations of bacteria can increase substantially when kept at too high temperatures for too long periods. This risk assessment demonstrated that the elderly had a greater risk of acquiring listeriosis, which justifies the current Finnish recommendation that the elderly should avoid eating CSS/SCS (Finnish Food Authority, 2019). As the elderly population consumes CSS/SCS more frequently and in greater amounts, it seems that either the recommendation not to eat these products has not reached this population or they have chosen to ignore it. Therefore, a better targeted and more effective way to educate the elderly would be needed. For example, the risk groups could be advised to store CSS/SCS in a way that prevents growth. Examples of successful education for specific population groups include the information given to pregnant women in public health care in Finland. Maternity and child health clinics are widely used by almost every pregnant woman and provide information on the recommended diet (The Ministry of Social Affairs and Health, n.d.).

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