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SCIENTIFIC REPORT OF EFSA

Analysis of the baseline survey on the prevalence of *Listeria monocytogenes* in certain ready-to-eat foods in the EU, 2010-2011 Part B: analysis of factors related to prevalence and exploring compliance[1]

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ABSTRACT

A European Union-wide baseline survey on *Listeria monocytogenes* was carried out in 2010 and 2011. Packaged (not frozen) hot or cold smoked or gravad fish, soft or semi-soft cheeses (excluding fresh cheeses) and packaged heat-treated meat products were sampled in 26 European Union Member States and in one country not belonging to the European Union. Multiple-factor analysis (Generalized Estimating Equations) was used to investigate the statistical association between several factors on which information was gathered during the baseline survey, and two outcomes: prevalence of *Listeria monocytogenes* and proportion of samples with counts exceeding 100 cfu/g, in the surveyed fish and meat products (no analysis is presented for cheese samples, owing to the small number of contaminated samples). Sparseness issues led to instability of the effect estimates for some of the factors. For fish samples, factors that exhibited a stable association with at least one of the two outcomes were 'Subtype of the fish product' (factor related to the type of processing), 'Number of antimicrobial preservatives and/or acidity regulators' and 'Possible slicing'. For meat products, the corresponding factors were 'Type of the meat product', 'Animal species of the origin of the meat product', 'Possible slicing' and 'Remaining shelf-life' (days between sampling and 'Use by date'). Furthermore, a statistical model was developed that allowed the use of estimates of the proportion of samples with an *L. monocytogenes* count > 100 cfu/g obtained from a single-unit sample survey of a population of RTE foods, in order to estimate the probability that if a five-unit sample had been taken from the same population, no individual unit, out of $n = 5$ units constituting the sample, would have exceeded the level of 100 cfu/g. The model was applied using data from the baseline survey for fish, cheese and meat products, at the end of shelf-life.

KEY WORDS

Listeria monocytogenes, multiple-factor analysis, prevalence, compliance, growth

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SUMMARY

A European Union-wide baseline survey on *Listeria monocytogenes* was carried out in 2010 and 2011 with the aim of estimating the European Union-level prevalence of *Listeria monocytogenes* in certain ready-to-eat (RTE) foods at retail. A total of 3 053 batches of packaged (not frozen) hot or cold smoked or gravad fish, 3 530 packaged heat-treated meat products and 3 452 soft or semi-soft cheeses were sampled from 3 632 retail outlets in 26 European Union Member States (MS) and one country not belonging to the European Union. Two fish product samples from the same batch were analysed upon arrival at the laboratory as well as at the end of shelf-life, whereas the meat products and the cheese samples were analysed at the end of shelf-life. All 13 088 food samples were examined for the presence of *L. monocytogenes*, in addition to the determination of the *L. monocytogenes* counts. European Union-level estimates of the prevalence and of the proportion of samples with *L. monocytogenes* counts for the fish samples at time of sampling and at the end of shelf-life as well as for cheese and meat samples at the end of shelf-life were presented in the Part A report, published by the European Food Safety Authority on 27 June 2013. The present report, Part B, provides the results of further statistical analysis of the baseline survey data.

The primary objective of the survey was to obtain valid EU-level estimates of prevalence and contamination levels of *L. monocytogenes* in the categories of surveyed RTE foods, by collecting and utilising comparable data from all MS, through harmonised sampling schemes. An important characteristic of the data from this baseline survey that greatly affected their further statistical analysis, which is the subject of the present report, is that, even though a large number of samples were obtained during the survey, the variety of the obtained samples was very large and the number of *L. monocytogenes*-contaminated samples and the number of samples with counts exceeding 100 colony forming units (cfu)/g were small. This affected especially the attempts at data analyses aiming at identifying factors related to the prevalence of contaminated foods and at developing predictive models for the microbial growth of *L. monocytogenes* under various storage conditions. In the former case, problems due to sparseness were evident during the model-building process, while no factor models are presented for soft or semi-soft cheese samples, owing to the very low number of cheese samples that were found to be contaminated with *L. monocytogenes* in the baseline survey. In the latter case, after extensive analysis of the available eligible data from pairs of fish product samples for the development of predictive models for the microbial growth of *L. monocytogenes*, it was concluded that, given the limitations of the available information and of the nature and characteristics of the collected baseline survey data, these data were not appropriate for the development of satisfactorily accurate predictive models. The above-mentioned issues limited the number and strength of conclusions that were possible from the further analysis of the data of the baseline survey.

Multiple-factor models were used to examine the statistical association between several factors, on which information was gathered during the baseline survey, and two outcomes:

a. Prevalence: a food sample was considered contaminated if *L. monocytogenes* was detected by at least one of either the detection or the enumeration methods (i.e. a sample was regarded as contaminated if 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).

b. Proportion of samples with an *L. monocytogenes* count that exceeded the level of 100 cfu/g.

Generalized Estimating Equations (GEE) methodology was used in order to account for the hierarchical nature of the baseline survey data. Six models were constructed: four models for fish product samples (for prevalence and for proportion of samples with an *L. monocytogenes* count that exceeded the level of 100 cfu/g, at time of sampling and at end of shelf-life) and two models for meat product samples (for prevalence and for proportion of samples with an *L. monocytogenes* count that exceeded the level of 100 cfu/g, at end of shelf-life). The results of the models are presented in the form of odds ratios (ORs) with corresponding 95 % confidence intervals (CIs) and *P*-values, estimated

each time for a specific category (or level) of a factor, compared with another category (or level) of the same factor.

To facilitate the implementation, interpretation and feasibility of statistical models, some additional variables were defined, and some existing categorical variables were redefined by collapsing some of their categories into new ones. The large number of factors affecting the modelled outcomes (prevalence and proportion of samples with an *L. monocytogenes* count > 100 cfu/g) in combination with the, frequently, very large variability in the characteristics of the food items and the great imbalance in the distribution of the food items among the levels of several factors, made the analysis difficult, owing to sparseness problems. This was greatly exacerbated by the small number of baseline survey samples that were positive for the examined outcomes, which led, on many occasions, to very unequal distribution of the data among the categories defined by the combinations of the levels of all factors and of the modelled outcomes in each model, including, on occasion, combinations with zero frequencies. These problems were evident during the model-building process and also 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 among different analyses. Care should be exercised when formulating statements about those factors that were unstable across different models. Therefore, the discussion of results 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.

The variable 'Subtype of the fish product' reflected the different types of processing that the fish products had undergone. The OR of being contaminated with *L. monocytogenes* for 'Hot smoked fish' and for 'Unknown smoked fish' (fish which may have been hot or cold smoked) compared with 'Cold smoked fish' was significantly lower than 1, meaning that the odds of a sample being contaminated with *L. monocytogenes* were significantly lower for 'Hot smoked fish' and for 'Unknown smoked fish' than for 'Cold smoked fish', both at time of sampling and at the end of shelf-life. Concerning the multiple-factor models for the proportion of samples with an *L. monocytogenes* count exceeding 100 cfu/g, the variable 'Subtype of the fish product' was not included in either of the two models (for time of sampling and for the end of shelf-life), as there was no significant association between this variable and that outcome. Concerning the 'Number of antimicrobial preservatives and/or acidity regulators (AP/AR)' the OR of being contaminated with *L. monocytogenes* for samples with 'Two or more AP/AR' were over seven times those of samples with 'No reported AP/AR', both at the time of sampling and at the end of shelf-life. Conversely, whilst not statistically significant, samples with 'One AP/AR' had lower odds of being contaminated with *L. monocytogenes* than samples with 'No reported AP/AR' both at sampling and at end of shelf-life. Furthermore, no significant association with the 'Number of antimicrobial preservatives and/or acidity regulators (AP/AR)' emerged in the model for the proportion of samples with *L. monocytogenes* counts in excess of 100 cfu/g. At the time of sampling, the odds of being contaminated with *L. monocytogenes* in 'Sliced fish' were 1.59 times the odds in 'Not sliced' fish (*P*-value = 0.04). While the odds of contamination in 'Sliced fish' remained higher than in 'Not sliced' fish products, at the end of shelf-life, that result was not statistically significant. The OR of having an *L. monocytogenes* count above 100 cfu/g for a 'Sliced' fish sample compared with a 'Not sliced' sample was 2.79 (*P*-value = 0.07) at the time-point of sampling and 2.55 (*P*-value = 0.03) at the end of shelf-life. In addition to this consistent and frequently significant association across the four models, this finding appeared to be robust in sensitivity analysis using weighted analysis. Several other factors were included in the final multiple-factor models for the fish samples, for at least one of the two outcomes; however, the results were not always stable in sensitivity analysis.

Concerning the models for the two outcomes for the meat products at the end of shelf-life, the most stable associations with the outcome were found for the following factors: 'Type of the meat product', 'Possible slicing', 'Animal species of the origin of the meat product' and 'Remaining shelf-life'. The OR of a sample being contaminated with *L. monocytogenes* for 'Pâté' compared to 'Cold, cooked meat product' was 2.91 (*P*-value = 0.005). However, 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). Furthermore, the odds of a sample being contaminated with *L. monocytogenes* for 'Sliced' meat products were 2.13 times the odds for 'Not sliced' meat products with a *P*-value of 0.07, while the odds of a 'Sliced' meat product sample having an *L. monocytogenes* count above 100 cfu/g were 2.61 times the odds for a 'Not sliced' meat product but were not significantly different (*P*-value = 0.36). Concerning the variable 'Animal species of the origin of the meat product' the OR of a meat product sample having an *L. monocytogenes* count above 100 cfu/g for 'All other species' compared with 'Avian species' was 0.35 (*P*-value = 0.04). Finally, the corresponding OR for a meat product sample compared with a sample whose 'Remaining shelf-life' is one day shorter was 1.010 (95 % confidence interval (CI): 1.005, 1.016) which was statistically significantly higher than 1 (*P*-value = 0.0002). Based on the results of the multiple-factor analysis it can be recommended that food business operators producing cold smoked fish, pâté or sliced ready-to-eat smoked or gravad fish and heat-treated meat products might actively reconsider food safety management systems and their ongoing verification, in particular with increased attention to environmental *L. monocytogenes* sampling in the area of the slicing process, in order to ensure effective control of *L. monocytogenes* in their products.

The final Term of Reference (ToR) for the work presented in this report, Part B, required the development of predictive models for compliance with *L. monocytogenes* food safety criteria in foods. Commission Regulation 2073/2005 mentions two microbiological criteria applicable for RTE foods at different stages. The criterion with which compliance might usefully be considered at the retail stage is the requirement for RTE foods not to harbour *L. monocytogenes* counts in excess of 100 cfu/g at the end of shelf-life. The fundamental requirement to predict compliance from this prevalence survey, therefore, involves consideration of what a survey of single-unit samples ($n = 1$) might represent for the surveyed population of RTE foods if a multiple-unit sample approach ($n = 5$) had been followed. In statistical terms, the probability of compliance for this exercise was defined as the probability that no individual unit, out of $n = 5$ units constituting a sample taken from a population of RTE foods, exceeds the level of 100 cfu/g, at the end of shelf-life. The estimation of this probability is based on an estimate of the proportion of samples with *L. monocytogenes* counts exceeding the level of 100 cfu/g obtained from a single-unit sample survey in the same population of RTE foods.

A statistical model was developed for this purpose and is presented in the current report. An illustration of the application and results of the developed model is provided by using the baseline survey data for fish, cheese and meat product samples. This method may have some utility when, for example, a Competent Authority has carried out a prevalence survey in a population of RTE foods, based upon a representative sampling plan, and wishes to make some assessment of compliance within that population of RTE foods. It has to be noted that the potential utility of such a statistical method would not alter the obligation on food business operators, which explicitly remains in Commission Regulation 2073/2005, to analyse $n = 5$ samples, in order to demonstrate compliance. The statistical methodology developed and applied in this report should not be seen as a way to facilitate demonstration of compliance by food business operators using fewer than five sample units.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Upon a request from the European Commission (EC), the European Food Safety Authority (EFSA) adopted a "Report of Task Force on Zoonoses Data Collection on proposed technical specifications for a survey on (*L. monocytogenes*) in selected categories of ready-to-eat food at retail in the EU (EFSA, 2009a)".

Based on one of the options in the EFSA proposals, the Commission adopted Decision 2010/678/EU of 5 November 2010[4] concerning a financial contribution from the Union towards a coordinated monitoring programme on the prevalence of *L. monocytogenes* in certain ready-to-eat foods to be carried out in the Member States. This large survey consisting of three subsurveys started on 1 January 2010 for a period of at least 12 months.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission requested EFSA on 14 February 2011, to analyse the results of the baseline survey on *L. monocytogenes* in certain ready-to-eat foods, in particular:

- to estimate the EU prevalence of *L. monocytogenes* in the surveyed ready-to-eat foods;
- to analyse the qualitative and quantitative survey test results;
- to analyse the factors related to the prevalence of contaminated foods;
- to develop predictive models for the microbial growth of *L. monocytogenes* under various storage conditions; and
- to develop predictive models for compliance with *L. monocytogenes* food safety criteria in foods.

[4] 2010/678/EU: 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 (notified under document C(2010) 7516). OJ L 292, 10.11.2010, p. 40-54.

ANALYSIS

1. Introduction

This report (Part B) describes the findings of statistical modelling analysis, of the results of a baseline survey carried out in the European Union (EU), to estimate the prevalence of *Listeria monocytogenes* in certain ready-to-eat (RTE) foods at the retail level. This study was the eighth in a series of baseline surveys carried out within the EU. It was the first baseline survey directly investigating foodstuffs at retail and it was also the first baseline survey enabling the estimation of the prevalence only at the EU level and not at Member State (MS) level. The primary objective of the survey was to obtain valid EU-level estimates of prevalence and contamination levels of *L. monocytogenes* in the categories of surveyed RTE foods, by collecting and utilising comparable data from all MS through harmonised sampling schemes. According to Article 5 of Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents[5], such surveys may be established, especially when specific needs are identified, to assess risks and to establish baseline values related to zoonoses and zoonotic agents. The results of such a survey should help inform consideration of the need for additional risk management strategies.

The retail survey was carried out over a two-year period, which commenced in January 2010. Examined foods were packaged (not frozen) hot or cold smoked or gravad fish, packaged heat-treated meat products, and soft or semi-soft cheeses, excluding fresh cheeses. Fish were analysed at the time of sampling (an arbitrary point in their shelf-life) and all three food categories were analysed at the end of shelf-life having been stored in the laboratory under refrigeration following retail sampling. The objectives, sampling frame, methods of bacteriological analysis, as well as the collection and reporting of data and the timelines of this baseline survey were specified in Commission Decision 2010/678/EU.

Coupled with the RTE nature of the foods sampled, and the quantitative component of the survey test results, this survey came much closer to the point of consumption than previous surveys. However, this survey targeted *L. monocytogenes* in RTE food products previously shown to be at risk of contamination, and did not consider consumption of surveyed products; thus it is not an exposure assessment. The rationale underpinning this targeting was that the EU Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the EU (EFSA, 2009b), which reports on ongoing official control monitoring, showed that the proportion of food samples exceeding the food safety criterion for *L. monocytogenes* in EU MS was highest in RTE fishery products, followed by RTE meat products and cheeses. According to the EU Summary Reports available when the study was designed, a significantly increasing trend in the notification rate of listeriosis cases in humans was observed between 2002 and 2006 (EFSA, 2007a). This notification rate remained at the same level in 2007, with 1 558 such cases registered in 26 MS (EFSA, 2009b). Illness was often severe and case fatality was reported at 20 %. In 2012, 26 MS reported 1 642 confirmed human cases of listeriosis and the EU notification rate was 0.41 cases per 100 000 population (EFSA and ECDC, 2014).

Consequently, the survey was not designed to examine the general exposure of EU consumers to *L. monocytogenes* in food, but targeted RTE food products previously shown to be at risk of contamination at levels considered to be a public health risk. Even within the food-groups sampled, the prevalences and quantities detected would need to be considered within the context of EU consumption patterns to enable any meaningful extrapolation to an EU exposure assessment.

An External Scientific Report prepared and submitted to EFSA by an EFSA contractor (later referred to as the External Report) (Rakhmawati et al., 2014) reports on the analysis of factors related to the

[5] 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.

prevalence of *L. monocytogenes*-contaminated foods and to the proportion of samples with counts exceeding 100 colony forming units (cfu)/g, as well as on the development and application of predictive models for the microbial growth of *L. monocytogenes* and for compliance with *L. monocytogenes* food safety criteria in foods. Therefore, this report might usefully be read in parallel with that External Report.

The EFSA Scientific Report, Part A (EFSA, 2013) reported on the analyses of the prevalence of *L. monocytogenes* in the surveyed RTE foods and of qualitative and quantitative survey test results. The present Part B Report presents the analyses of factors related to the prevalence of contaminated foods and the development of a predictive model for compliance with *L. monocytogenes* food safety criteria in foods. No results are presented for the development of predictive models for the microbial growth of *L. monocytogenes* under various storage conditions, for reasons that will be explained later in the report.

Twenty-six EU MS, i.e. all except Portugal, participated in the survey. In addition, one country not belonging to the EU, Norway (later referred to as a non-MS), participated in the survey. Results presented in this Part B report are based on the data reported by all participating countries for the multiple-factor statistical models, while only MS data were used for the examples of the use of the predictive model for compliance.

2. Objectives

The primary aim of the survey was to estimate the EU prevalence of *L. monocytogenes* in the following RTE food categories, in samples selected at random at retail level: packaged (not frozen) hot or cold smoked or gravad fish, packaged heat-treated meat products, and soft or semi-soft cheeses, excluding fresh cheeses. The previously published Part A report described the results of the analyses of the EU prevalence of *L. monocytogenes* in the surveyed RTE foods and the analysis of the qualitative and quantitative survey test results.

The specific objectives related to this Part B report were the following:

- analysis of factors related to the prevalence of contaminated foods,
- development of predictive models for the microbial growth of *L. monocytogenes* under various storage conditions, and
- development of predictive models for compliance with *L. monocytogenes* food safety criteria in foods.

Due to the specific design of the survey, these objectives were relevant only at the EU level and not at the MS level.

3. Materials and methods

A detailed description of the design of the survey can be found in Commission Decision 2010/678/EU. The sampling design, analytical methodology and sample size are described in Annexes I and II of that Decision.

3.1. Survey design

Sample size considerations for the baseline survey are described in the "Report of the Task Force on Zoonoses Data Collection on proposed technical specifications for a survey on *L. monocytogenes* in selected categories of RTE food at retail in the EU" (EFSA, 2009a) and in the EFSA Report Part A (EFSA, 2013). The number of samples to be taken per RTE food category in each MS was set out in Annex II of Commission Decision 2010/678/EU. In each MS, a multistage cluster sampling design was used, considering three levels of sampling: major cities/towns, retail outlets and the food product category (among the three product categories sampled: smoked or gravad fish, soft or semi-soft

cheeses, and heat-treated meat products). The actual RTE foods, within the three RTE food categories were selected based on the marketing data and detailed in the national sampling plan. Samples were selected by MS authorities at random at retail level based on their availability in the retail outlets. Concerning smoked or gravad fish, two separately packaged samples were to be taken from each sampled batch. One of these two samples should be analysed on the day of receipt of the sample at the laboratory and the other at the end of shelf-life. For soft and semi-soft cheeses and heat-treated meat products, only one sample should be taken from a batch in order to be analysed at the end of shelf-life.

Samples were taken at random from the customer display and were to weigh at least 100 g each. Only packaged and intact (sealed) packages, packaged by the manufacturer, were to be collected for sampling. However, in the case of cheeses and meat products, products packaged at the retail outlet could also be collected for sampling. Detection and enumeration analyses of *L. monocytogenes* were made at the end of shelf-life for all three types of the surveyed RTE foods and, also, at the time of sampling for the packaged fish samples.

Data on the following characteristics of the samples were collected using a mandatory questionnaire filled out by the competent authorities, or under their supervision, at the time of sampling and on arrival at the laboratory. Some additional (optional) data and variables were provided on a voluntary basis by MS:

(a) For all samples: 'Country', 'Code of the town', 'Code of the retail outlet', 'Type of retail outlet', 'Date of sampling', 'Type of sample' ('Soft/semi-soft cheese', 'Smoked or gravad fish' or 'Heat-treated meat product'), 'Reference of the sample', 'Comment' (optional), 'Possible slicing', 'Packaging type', 'Use by date', 'Production date' (optional), 'Packaging date' (optional), 'Country of production', 'Storage temperature at retail', 'Transport protocol', 'Date of testing at the end of the shelf-life (starting time)', '*Listeria monocytogenes* quantification result at the end of the shelf-life', '*Listeria monocytogenes* detection at the end of the shelf-life', 'Storage temperature at laboratory up to the end of shelf-life', 'Suitability for human consumption at end of shelf-life' (optional).

(b) In addition to (a), for samples of the type: 'Heat-treated meat product': 'Animal species of the origin of the meat product', 'Type of meat product' ('Sausage, Pâté, 'Cold, cooked meat product') and 'Packaging place for meat'.

(c) In addition to (a), for samples of the type: 'Smoked or gravad fish': 'Subtype of the fish product' ('Cold smoked fish', 'Hot smoked fish', 'Unknown smoked fish', 'Gravad fish'), 'Fish species', 'Preservatives and acidity regulators', 'Date of testing for fish product on the arrival at the laboratory (starting time)', '*Listeria monocytogenes* quantification on the arrival at the laboratory', '*Listeria monocytogenes* detection on the arrival at the laboratory', 'pH test result on the arrival at the laboratory', 'Water activity (a_w) result on the arrival at the laboratory'.

(d) In addition to (a), for samples of the type: 'Soft/semi-soft cheese': 'Subtype of cheese' ('Smear-ripened', 'Mould-ripened', 'Brine-matured', 'Otherwise ripened', 'Unknown'), 'Type of milk treatment' ('Raw milk', 'Thermised milk', 'Pasteurised milk', 'Unknown'), 'Animal origin of the milk' ('Cow', 'Sheep', 'Goat', 'Buffalo', 'Mixed', 'Unknown'), 'Packaging place for cheese', 'Cheese rind included in the analysis', 'Percentage of rind' (optional).

The data dictionary, for the *L. monocytogenes* baseline survey can be found in Appendix A.

3.2. Data description

A detailed description of the validation and cleaning of the dataset carried out was provided in the Part A report (EFSA, 2013). The final validated dataset included information on a total of 13 088 samples, sampled from 3 632 retail outlets in 26 MS and Norway. It comprised 3 053 smoked or gravad fish samples on arrival at the laboratory and 3 053 smoked or gravad fish samples at the end of shelf-life,

3 530 heat-treated meat products at the end of shelf-life and 3 452 soft/semi-soft cheese products at the end of shelf-life. Portugal did not submit data. This validated dataset formed the basis for all subsequent analyses. Statistical analysis of factors related to the prevalence of contaminated foods, along with the corresponding descriptive information and statistics, presented in this Part B report, include also the data submitted by Norway; therefore, some numerical information, e.g. prevalence, differed from the Part A report, in which Norwegian data were not included. The results presented in this Part B report are based on the data reported by all participating countries for the multiple-factor statistical models, while only MS data were used for the application of the predictive model for compliance.

3.3. Analysis of factors related to the prevalence of contaminated foods

The effect of factors potentially associated with *L. monocytogenes* prevalence and with the proportion of samples with an *L. monocytogenes* count that exceeded the level of 100 cfu/g was investigated, using Generalized Estimating Equations (GEE) (Liang and Zeger, 1986). Multiple-factor models were constructed for smoked or gravad fish samples at time of sampling and at the end of shelf-life and for packaged heat-treated meat products at the end of shelf-life. No models are presented for soft or semi-soft cheese samples, owing to the very small number of samples that were found to be contaminated with *L. monocytogenes* in the baseline survey (for details see EFSA Report Part A — EFSA, 2013). All countries that participated in the survey (MS and Norway) were included in this analysis.

3.3.1. Definition of the outcome variables

The analyses were performed for two different outcome variables:

a. 'Prevalence': this variable was based on combined results of the detection and enumeration methods. A food sample was considered contaminated if the presence of *L. monocytogenes* in it was evidenced by at least one of either the detection or the enumeration method (i.e. a sample was regarded as contaminated when 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). As the potential for false-positive results is low with both methods, and non-homogeneous bacterial distribution might well account for discordance, particularly in samples containing low *L. monocytogenes* counts, any positive result was regarded as indicating that the sample was contaminated.

b. 'Proportion of samples with an *L. monocytogenes* count that exceeded the level of 100 cfu/g'.

For fish product samples, separate multiple-factor analysis was conducted for the outcomes at time of sampling and at the end of shelf-life while for meat product samples multiple-factor analysis is presented for the two outcomes at the end of shelf-life.

3.3.2. Factors investigated

The joint association between the outcome variables and potentially associated factors on which information was gathered in the baseline survey was examined, for fish and meat samples. Main effects and two-way interaction effects were included in the full models, which were reduced to the final models using model selection techniques. Some additional (optional) data and variables were collected on a voluntary basis by MS. However, the effects of these optional factors could not be evaluated due to the scarcity and/or imbalance of responses of the data reported.

To facilitate the implementation, interpretation and feasibility of statistical models, some additional variables were defined, and some existing categorical variables were redefined by collapsing some of their categories into new ones. These are described in the following sections.

3.3.2.1. EC 2073/2005 not-supporting growth variable

Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs[6] sets out different microbiological criteria for RTE foods supporting the growth of *L. monocytogenes* and RTE foods not supporting the growth. This Regulation then defines some characteristics of products which would automatically place RTE foods in the category of 'not supporting growth'. Foods other than those may well also not support *L. monocytogenes* growth. For the purposes of this analysis, a variable was defined to indicate whether a specific fish sample could be considered to be 'automatically in the category of not supporting the growth (NSG)', based on Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. This binary variable, referred to, henceforth, as 'EC 2073/2005 NSG' variable, has two levels: 'Included in EC 2073/2005 NSG' and 'Not included in EC 2073/2005 NSG'. These are defined (for the present study) by the following thresholds:

If for a sample:

- the pH was less than or equal to 4.4, or,
- the water activity (a_w) was less than or equal to 0.92, or
- the pH was less than or equal to 5 and the water activity was less than or equal to 0.94,

the variable 'EC 2073/2005 NSG' was assigned the value 1 ('Included in EC 2073/2005 NSG'), otherwise it took the value 0 ('Not included in EC 2073/2005 NSG'). This variable could not be assessed for a food item at the instant the sample was taken; however, it was based on the determination of the water activity and pH of the sample tested on arrival at the laboratory. In the current baseline survey, 210 packaged fish samples fulfilled at least one of the above pH and/or a_w criteria, and, therefore, were included in the category 'Included in EC 2073/2005 NSG'. For more details the reader is referred to the EFSA Report, Part A (EFSA, 2013). Since the assignment of samples into the two levels of the 'EC 2073/2005 NSG' variables was based on their pH and a_w measurements, these two characteristics were not considered for inclusion in the models separately. Moreover, for the fish sample models at the end of shelf-life, the same values of the 'EC 2073/2005 NSG' were used in the analysis, even though the determination of pH and a_w was done only at time of sampling.

Additionally, the probability of *L. monocytogenes* growth can be estimated based on food intrinsic and extrinsic factors. For example, Tienungoon et al. (2000) modelled the probability of growth as a function of pH, water activity and temperature. This model was used to create a continuous variable that expressed the probability of no-growth (1 - *P*) of *L. monocytogenes* in the RTE food items used in the present analysis. The values for the parameters $b_0 - b_7$ were based on estimated ranges for *L. monocytogenes* strain Scott A (Tienungoon et al., 2000). If any of the temperature, water activity or pH values was below their minimal values used in the model ($T_{min} = 0.4164$, $a_{w\,min} = 0.9142$, $pH_{min} = 3.35$), the continuous variable expressing the probability of no-growth was taken equal to 1. It has to be noted that this is a broth-based model and, hence, it might overestimate the probability of *L. monocytogenes* growth in the RTE food items used in the present analysis. Whilst the main conclusions of the analysis are based upon models that considered the binary 'EC 2073/2005 NSG' variable, the continuous no-growth variable has been included, instead of the binary variable 'EC 2073/2005 NSG', in the final models in the form of a sensitivity analysis.

3.3.2.2. Fish species

As explained in the EFSA Scientific Report, Part A (EFSA, 2013), the reported information on the species of fish in the products sampled for the baseline survey, was summarized by classifying the data in the following five categories: 'Salmon', 'Herring', 'Mackerel', 'Mixed fish' and 'Other fish'. The same classification was used in the current analysis for the 'Fish species' variable.

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

3.3.2.3. Packaging type

The original 'Packaging type' variable (with four categories: 'Vacuum', 'Modified atmosphere', 'Normal atmosphere', 'Other') caused sparseness problems (see Section 3.3.5.5 and the External Report) in some of the final models and it was necessary to collapse it to a binary version (two levels, 'Modified atmosphere' and 'All other packaging types', the latter including also 'Normal atmosphere' and 'Vacuum'-packed samples, as well as samples in the category 'Other'). To have a consistent presentation of the final models, it was decided to replace the original 'Packaging type' variable in all final models by its modified binary version.

3.3.2.4. Type of retail outlet

The original variable 'Type of retail outlet' (with four categories: 'Supermarket or small shop', 'Street market/farmers' market', 'Speciality delis', 'Other') caused sparseness problems in some of the final models as most samples were obtained in 'Supermarket or Small shop' and collapsing the variable to a binary version (two levels, 'Supermarket or small shop' and 'All other types of retail outlet') was the approach taken in all final models.

3.3.2.5. Animal species of the origin of the meat product

The original variable 'Animal species of the origin of the meat product' had seven possible values listed in the data dictionary: 'Pork', 'Beef', 'Turkey', 'Broiler', 'Poultry', 'Mixed' and 'Other'). One sample was reported as originating from 'Goose'. Consideration of the variable in its original form caused sparseness problems in some of the final models and, therefore, it was necessary to create a binary version of the variable by collapsing its values into two levels: 'Avian species' (including 'Turkey', 'Broiler', 'Poultry' and 'Goose') and 'All other species' (including 'Pork', 'Beef', 'Mixed' and 'Other').

3.3.2.6. Sampling season

The variable 'Date of sampling' was transformed into 'Sampling season', where 'Winter' represents the months December, January and February, 'Spring' comprises March, April and May, 'Summer' comprises June, July and August and 'Autumn' is September, October and November.

3.3.2.7. Remaining shelf-life

The 'Remaining shelf-life' was defined as the difference between the final date for using the product (as labelled 'use by date') and the date of collection of the sample ('date of sampling'), the latter date being an arbitrary point during the shelf-life of the product.

3.3.2.8. Number of antimicrobial preservatives and/or acidity regulators (AP/AR)

Analysis of the data reported for the variable 'Preservatives and acidity regulators' revealed that a total of 47 different combinations of food additives and ingredients other than fish flesh had been recorded in the fish samples dataset. In order to classify the sampled fish products, the modified variable 'Number of antimicrobial preservatives and/or acidity regulators (AP/AR)' was defined, based on eight groups of reported additives, all of which possess an E-number, and all of which are known to act as preservatives and/or acidity regulators in processed fish. This variable had three levels, referring to the number of reported preservatives and acidity regulators: 'No reported AP/AR', 'One AP/AR', 'Two or more AP/AR'. Some of the reported ingredients neither possess known antimicrobial properties nor function as acidity regulators and, consequently, the respective food items were classified in the category 'No reported AP/AR'. In addition, food items with reported natural ingredients with direct or indirect antibacterial properties e.g. salt, sugar, smoke or herbs (if/when reported on the label and if/when recorded by sampling personnel), were also included in this category, as well as those for which 'Unknown' or 'Other' was reported for the original 'Preservatives and acidity regulators' variable. The detailed rationale for exclusion of specific reported ingredients and for the categorisation of fish samples according to reported additives is included in EFSA Report Part A (EFSA, 2013).

3.3.3. Descriptive analysis

Descriptive analysis concerning the factors appearing in the final models for both outcome variables for fish and meat samples, can be found in Appendix B; further descriptions of the fish and meat samples can be found in the External Report (Rakhmawati et al., 2014).

3.3.4. Exploratory bivariable analysis of potentially associated factors

In order to take into account the hierarchical structure in the dataset (country, city and retail outlet), Generalized Estimating Equations (GEE) methodology (Liang and Zeger, 1986), with independence correlation structure, was used to study the association between the outcome variables and the individual potentially associated factors, for fish and meat samples. This analysis appears in the External Report. For fish products, the 'EC 2073/2005 NSG'variable, as well as its interaction with the potentially associated factor, was also included in these models. So in this situation, the 'single-factor model' sections refer to models with one factor in addition to the 'EC 2073/2005 NSG'variable (and their interaction). More details and results of this analysis can be found in the External Report. Owing to possible confounding[7] these results should be interpreted cautiously and only within the context of an exploratory analysis.

3.3.5. Identification of factors associated with the modelled outcomes

The joint association between the outcome variables and potentially associated factors on which information was gathered in the baseline survey was examined by fitting multiple-factor regression models. Main effects and the respective two-way interaction effects were included in the full model, which was reduced to a final model using model selection techniques. Main inferences were made using Generalized Estimating Equations (GEE), which is a valid methodology for inference with hierarchical and clustered data. In this analysis the data were organised in three levels of hierarchy: retail outlets nested in cities/towns, and cities/towns nested in countries. The independence correlation structure was applied. The specific model-building approach that was followed is described in Appendix C and is detailed further in the External Report.

The main effect of the 'EC 2073/2005 NSG' variable was always included in the final models for the fish products, even if it was not significant, in order to determine whether being automatically included in this category could have an effect on the modelled outcome or on the effect (odds ratio, OR) of other factors on the modelled outcome. The same approach was also followed for the main effect of the variable 'Possible slicing', both for fish and meat products, because reports in the scientific literature indicate that slicing of a food item can be related to the modelled *L. monocytogenes*-related outcomes. The interaction terms between 'Packaging type' and 'Storage temperature at retail' as well as between 'Packaging type' and 'Storage temperature at laboratory up to the end of shelf-life' (as well as the respective main effects) were initially included in the multiple-factor models for fish and meat samples (for products tested at time of sampling and at end of shelf-life, respectively), since they were considered to be potentially biologically relevant. The reasoning is that the presence of increased concentrations of CO_2 in the modified packaging atmosphere may impact on the growth potential and thus, indirectly, on the probability of detection of *L. monocytogenes* in a sample, while also the water solubility of CO_2 increases if the temperature drops, thus enhancing the antimicrobial effect (Devlieghere et al., 2001). Whenever this interaction was not statistically significant it was not retained in the final multiple-factor models, presented in this report, Part B (in which case, the respective main effects were also evaluated separately concerning their retention in the final models). The multiple-factor models which include this interaction, even in

[7] In bivariable analysis, a potential risk factor might appear to be associated with the outcome solely because of its association with another risk factor. Confounding is, therefore, the over- or under-estimation of the effect of a potential risk factor due to its association with other risk factors. In order to eliminate confounding, and to obtain valid estimates of the effect of risk factors, an adjustment for the confounding variable is necessary, which can be achieved by multivariable regression analysis. In certain cases, however, two or more potential risk factors may be so strongly associated that separate estimates of their effects cannot be obtained. In this case, the term collinearity or multicollinearity is used.

cases in which it was not significant (as well as the respective main effects), can be found in the External Report.
Throughout this scientific report the level of significance for hypotheses testing was set to 0.05. All confidence intervals (CIs) are constructed with 95 % coverage probability.

3.3.5.1. Interpretation of model findings—odds ratios

The associations between the outcome variables and the potentially associated factors are indicated in the form of ORs. An OR is a measure of association between an exposure or risk factor and a binary outcome. It is the ratio of the odds of a positive outcome under two different conditions for the factor. The odds is the ratio $\pi/(1-\pi)$ of the probability that an event will occur to the probability that it will not occur. It is different from the risk, π, which is simply the probability that the event will occur. In the same way an OR is the ratio of two odds, whereas the relative risk is the ratio of two risks. In case of a (very) rare phenomenon (π being small), both are approximately equal, as in that case the denominator in an OR $(1-\pi)$ is close to 1. More details on the interpretation of modelled associations can be found in Appendix C.

The regression coefficients (βs) are related to the ORs as follows: the exponentiated value of a regression coefficient equals to the OR associated with a one-unit increase in the exposure factor. This applies to factors that do not participate in any interaction terms. When interaction is present, the OR between a particular factor and the outcome varies according to, and depends upon, the value of the other factor involved in the interaction term. If the interaction between factors is significant, the main effects are no longer summarising the effect of the factors. Indeed, in these cases, the effect of one factor varies with the value of the other factor, and one needs to look at the main effects together with the interaction effect. In those situations, it is common practice to keep also the main effects in the model even if they are not significant.

3.3.5.2. Analysis of multicollinearity among potentially associated factors

Highly intercorrelated factors may cause multicollinearity problems when included together in regression models. Such models may get computationally unstable and inference may become spurious. Multicollinearity was assessed in the final multiple-factor models, by computing the Variance Inflation Factor (VIF), which was used as a formal method to detect correlation among factors. The VIF measures how much the variances of the estimated regression coefficients are inflated compared with when the factors are perfectly unrelated.

A VIF value that equals 1 indicates that there is no correlation among factors, whereas VIF values greater than 1 indicate some correlation. VIF values exceeding 10 are interpreted as an indication of strong multicollinearity and consequently of potential problems.

3.3.5.3. Goodness-of-fit

In order to check the goodness-of-fit of the final model, the test of Hosmer and Lemeshow (Agresti, 2013) was applied. However, the test was developed for logistic regression only and not for GEE or Firth's method. Consequently, it is not known what effect the clustered nature in combination with the sparseness of the data has on the validity of the test. Therefore, findings of these goodness-of-fit tests need to be interpreted with caution in the present context. More details on this issue can be found in the External Report.

3.3.5.4. Sensitivity analysis

To get further insight into the stability of the final models, their sensitivity to some modifications was investigated. More specifically, the following analyses were used:

- Weighted estimation: while the allocation of the number of samples to be taken in each MS was made approximately according to the size of the human population of each MS, further analysis was undertaken, using two different sets of weights, correcting for over- or under-

representation of certain participating countries in the baseline survey sample. The weights that were used were based on the planned sample size (a weight corresponding to a planned sample size of 0.5 was used for Norway) and on the size of the human population of each country. Unweighted as well as weighted estimation was considered for all final models, in order to examine the sensitivity of the estimates (ORs) and corresponding standard errors to corrections for the non-optimally achieved sampling schemes. For additional details the reader is referred to the External Report.

- The method of Firth (1993) applied to logistic regression: yields a bias correction in case the maximum likelihood (ML) estimates might be biased, e.g. when probabilities to be estimated are very small.
- Exact logistic regression (Hirji et al., 1987): provides exact inference as an alternative to asymptotic inference, in case the latter might not be valid, e.g. when probabilities to be estimated are very small. Exact logistic regression was performed whenever feasible (for more details the reader is referred to the External Report). Both exact logistic regression and the method of Firth are methods that can cope with sparseness, to some extent.
- For fish product samples, the continuous no-growth probability was used instead of the 'EC 2073/2005 NSG' variable.

For more details, the reader is referred to the External Report. A summary of the sensitivity analysis results for the final models can be found in Appendix D.

3.3.5.5. Sparseness

Sparseness is a well-known phenomenon complicating the analysis of categorical data. At a basic level, sparseness is apparent even in the basic chi-square test for dependency between two (categorical or categorised) variables. In cases where the expected frequencies are less than 5 in one of the inner cells of the cross-classification table of the two variables, the asymptotic chi-squared null distribution of the Pearson or deviance test statistic for testing the null hypothesis of independence becomes questionable. As the expected frequency of a cell equals the total sample size multiplied by the probability for that particular cell, sparseness manifests itself when the sample size is too small in relation to the probability for that cell. In other words, the sample size needs to be large enough in combination with a cell probability that is not too small. This same issue appears in models for categorical outcomes, such as logistic regression and extensions thereof, and it might affect inference through biased estimates as well as through invalid asymptotic distributions for these estimates and for the (null) distribution of test statistics such as the Wald test, the likelihood ratio test or the score test.

The higher the number of categorical covariates appearing in a logistic-type regression model, the more likely it is that this issue arises. Indeed, more categorical covariates and interaction effects thereof are equivalent to the analysis of more dimensional tables, spreading the (fixed) number of observations among more and more cells in such tables. More cells defined by combination of values of more and more covariates will be populated by fewer and fewer observations and, thus, will lead to erroneous or misleading estimates and conclusions from hypothesis tests. In the current analysis, the issue of sparseness arose from the multitude of investigated factors in combination with the, frequently, very large variability in the characteristics of the food items and the great imbalance in the distribution of the food items among the levels of several factors. It was greatly exacerbated by the small number of baseline survey samples that were positive for the examined outcomes (*L. monocytogenes* prevalence and proportion of samples with *L. monocytogenes* counts exceeding 100 cfu/g), which led, on many occasions, to very unequal distribution of the data among the categories defined by the combinations of the levels of all factors and of the modelled outcomes in each model, including, on occasion, cells which contained no data at all.

Some more details and an example illustrating sparseness in the context of the current analysis can be found in the External Report.

3.4. Predictive models for the microbial growth of *Listeria monocytogenes* under various storage conditions

The development of predictive models for the microbial growth of *L. monocytogenes* requires data on the pathogen's cell density at different storage times and under various storage conditions. The eligible data derived from the baseline survey as well as the appropriateness of these data for the development of predictive models are discussed below.

3.4.1. Eligible dataset for development of predictive models

Data of a fish products sample pair were considered to be 'eligible' to be used in the development of predictive models if:

- the outcome at end of shelf-life was at or above the detection limit of the enumeration method (10 cfu/g);
- the outcome at end of shelf-life was not less than that at time of sampling, i.e. the count did not decrease;
- the date of testing at the end of shelf-life was beyond the date of testing at time of sampling, i.e. both samples could not have been tested on the same day.

In the complete dataset of the baseline survey for the fish products under consideration, a total of 2 923 pairs of fish samples did not have *L. monocytogenes* counts ≥ 10 cfu/g at both testing times. Only 35 of the total 3 053 pairs of fish samples from the same batch were found to have an *L. monocytogenes* count ≥ 10 cfu/g both at the time of sampling and at the end of shelf-life. Among those, 15 pairs of samples had a lower *L. monocytogenes* count at the end of shelf-life than at time of sampling (Figure 1) and thus provided no 'eligible' data for development of predictive models. Among the remaining 20 pairs of samples (out of the 35), 18 had a count at the end of shelf-life higher than that at time of sampling and two had equal *L. monocytogenes* counts at both times (Figure 2). Additionally, 64 pairs of samples had an *L. monocytogenes* count ≥ 10 cfu/g at the end of shelf-life but less than 10 cfu/g at time of sampling (34 out of these 64 were positive by detection at time of sampling, while 30 were negative by detection at time of sampling) but in one of these 64 pairs both samples were tested on the same day, the 'use by date' (Figure 3). Finally, 31 pairs of samples had an *L. monocytogenes* count ≥ 10 cfu/g at the time of sampling but less than 10 cfu/g at the end of shelf-life (Figure 1).

In conclusion, the *L. monocytogenes* count was non-decreasing for 84 pairs of samples; however, in one of these sample pairs both samples were tested on the same day, thus restricting the actual 'eligible' dataset for development of predictive models to 83 pairs of samples.

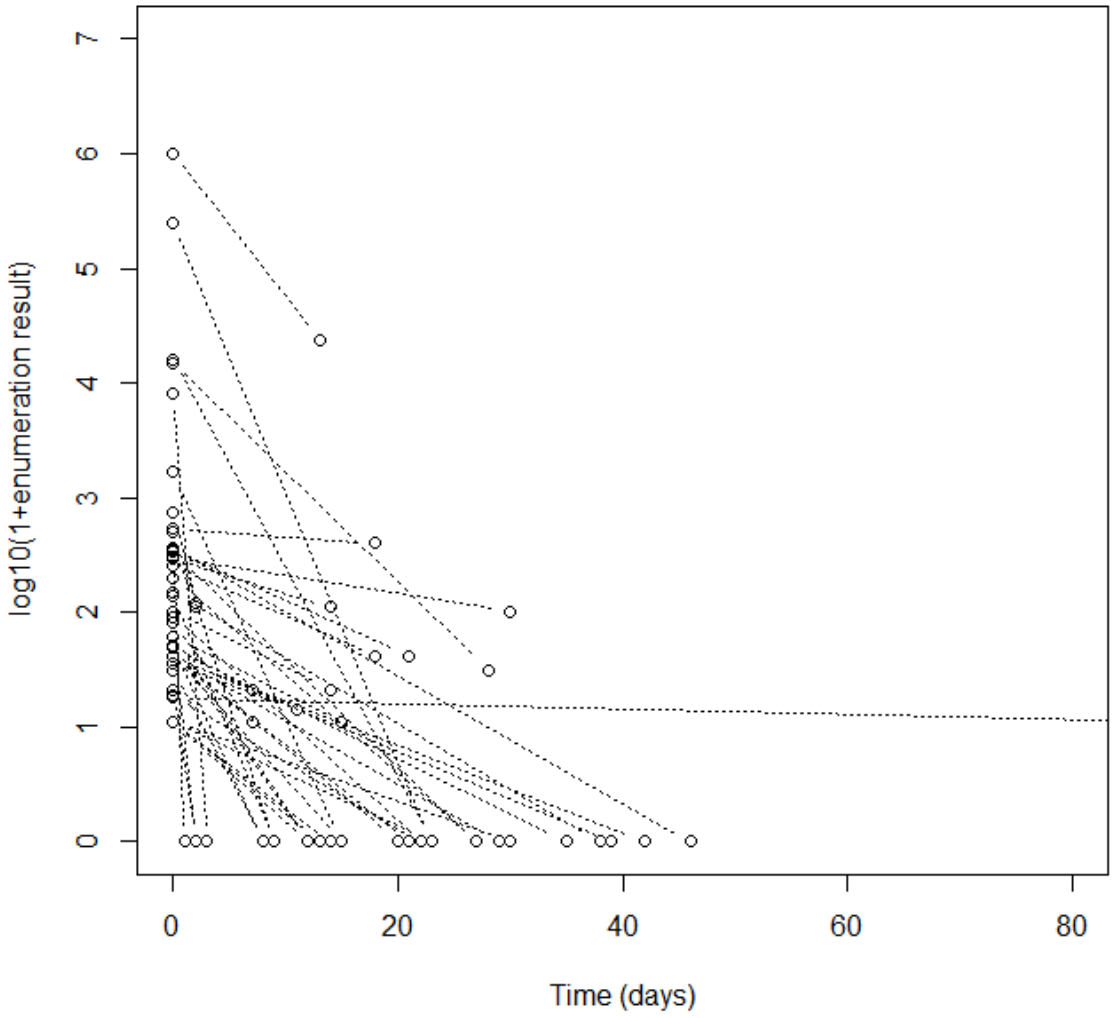

Figure 1: Graphical representation of the sample pairs with a decreasing *Listeria monocytogenes* count (46 sample pairs). Time of testing is presented on the x-axis as zero for samples tested at time of sampling. The plotted time of testing for the sample tested at the end of shelf-life is the number of days since the testing of the first sample of the pair at time of sampling. Concerning the samples with counts < 10 cfu/g, the count was considered to be zero for the purposes of this figure. In all three figures of counts (Figures 1, 2 and 3) the logarithms of the counts are plotted after adding 1 to all counts, in order to be able to plot '0' counts.

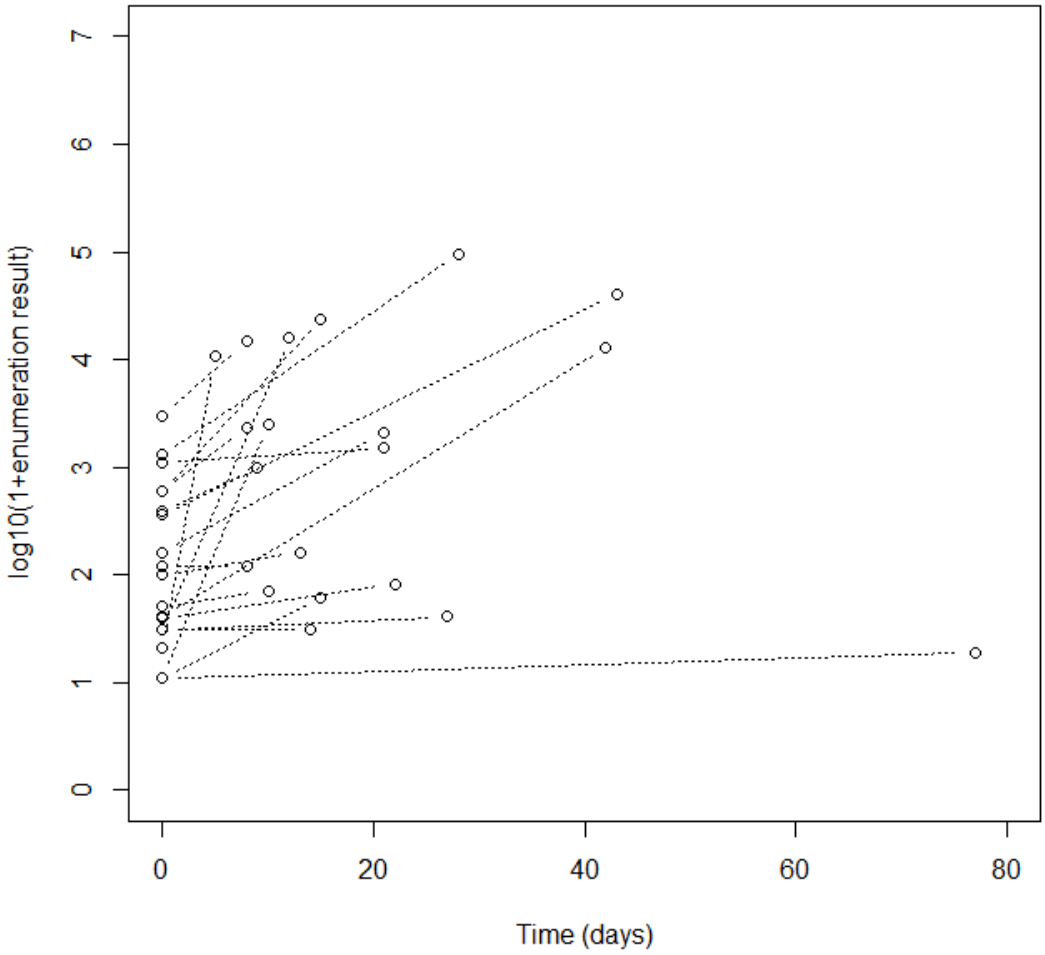

Figure 2: Graphical representation of the sample pairs with a non-decreasing *Listeria monocytogenes* count and an initial count ≥ 10 cfu/g (20 sample pairs). Time of testing is presented on the x-axis as zero for samples tested at time of sampling. The plotted time of testing for the sample tested at the end of shelf-life is the number of days since the testing of the first sample of the pair at time of sampling. In all three figures of counts (Figures 1, 2 and 3) the logarithms of the counts are plotted after adding 1 to all counts, in order to be able to plot '0' counts.

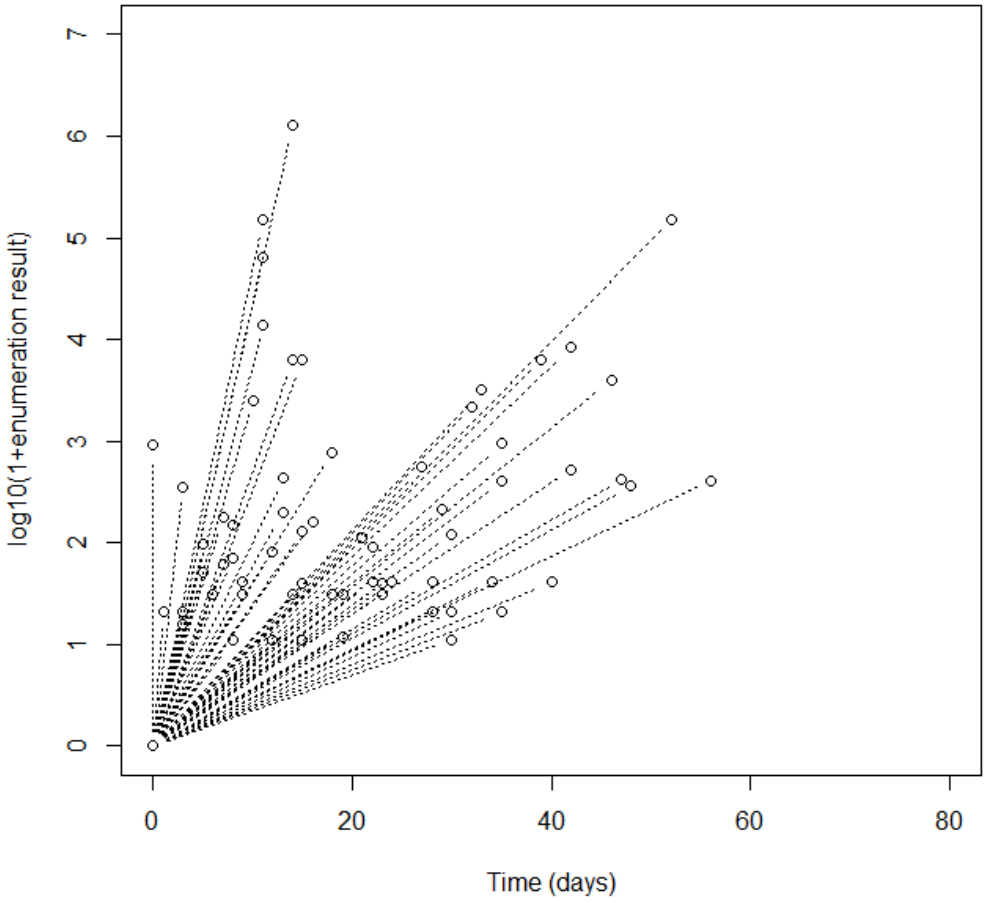

Figure 3: Graphical representation of the sample pairs with a non-decreasing *Listeria monocytogenes* count and an initial count < 10 cfu/g (64 sample pairs). Time of testing is presented on the x-axis as zero for samples tested at time of sampling. The plotted time of testing for the sample tested at the end of shelf-life is the number of days since the testing of the first sample of the pair at time of sampling. Concerning the samples with counts < 10 cfu/g, the count was considered to be zero for the purposes of this figure. In all three figures of counts (Figures 1, 2 and 3) the logarithms of the counts are plotted after adding 1 to all counts, in order to be able to plot '0' counts.

3.4.2. Appropriateness of eligible data for the development of predictive models

After extensive analysis of the available eligible data from the 83 pairs of fish product samples it was concluded that, given the limitations of the available information and of the nature and characteristics of the collected data, these data were not appropriate for the development of satisfactorily accurate predictive models for the growth of *L. monocytogenes.* The reasons are described in detail below:

- **Limited number of positive samples**: Since only data from samples with *L. monocytogenes* counts ≥ 10 cfu/g could be used in the development of a predictive model for *L. monocytogenes* growth, the low prevalence of the pathogen found in the tested food products was an important obstacle in developing a growth model. For example, only 20 of the total 3 053 pairs of fish product samples had counts ≥ 10 cfu/g at both testing times, while in 64 pairs the pathogen count exceeded (or was equal to) the level of 10 cfu/g at the end of shelf-life but had an *L. monocytogenes* count < 10 cfu/g at the time of sampling. Considering the increased number of the environmental factors (temperature, pH, a_w, presence and concentration of several antimicrobials, packaging atmosphere, numbers and type of competing microflora) that affect microbial growth and should be included in

the model, the above data are very limited for the development of a model with an acceptable performance.

- **Limited number of points in the growth curve**: One of the steps in the development of a predictive model is the use of primary models to estimate the kinetic parameters such as the growth rate and the lag phase. The quality and the number of the data points in the growth curve can affect significantly the accuracy of the estimation of the above kinetic parameters. In the case of the baseline survey, only two points (testing at time of sampling and at the end of shelf-life) were available. In this case, the lag phase cannot be estimated, and this may also lead to an erroneous estimation of the growth rate.
- **Sampling from different packages**: In the baseline survey, two fish samples from two distinct, intact packages from the same batch were collected; one was tested at the time of sampling and the other at the end of the shelf-life. It is not known what the exact relation between the two samples is. Considering the heterogeneity in the distribution of *L. monocytogenes* within a contaminated batch, it is not clear if the sample at the end of shelf-life gives an accurate representation of the sample at time of sampling, after having been kept in the laboratory under refrigeration for the remaining shelf-life. In addition, for the 64 fish sample pairs that had an *L. monocytogenes* count ≥ 10 cfu/g at the end of shelf-life, but for which the pathogen was absent (count < 10 cfu/g) at the time of sampling, the 'true' concentration of the pathogen at time of sampling is uncertain. This uncertainty in the concentration at the time of sampling can affect significantly the estimated growth rate for these sample pairs.
- **Unknown production time**: The problem with the uncertainty concerning the 'true' concentration of the pathogen at the time of sampling for the samples with *L. monocytogenes* counts ≥ 10 cfu/g at the end of shelf-life described above could be overcome by taking the time of production as zero time and assuming that the concentration of *L. monocytogenes* at that time is expected to be very low since, for most products, contamination with the pathogen occurs at processing stages post heat treatment. For such a low contamination level, the heterogeneity in the extent of contamination is not expected to affect significantly the estimation of the growth rate. In the baseline survey the time of sampling at retail is an arbitrary point during the shelf-life of the product, and the production time of the tested samples was not recorded for most samples. Since the production time can affect significantly the estimation of the growth rate, any assumption on this factor would lead to a high uncertainty.
- **Unknown concentration of antimicrobials**: The concentration of antimicrobial preservatives and/or acidity regulators can influence significantly the growth of *L. monocytogenes*. However, such information was unknown for most of the samples. Additionally, natural antimicrobials were sometimes included in some of the surveyed food items, as part of the production process (e.g. smoke), and these would be very difficult to describe/account for in this analysis.
- **Missing information on the background flora**: Microbial interactions can be an important factor controlling growth of pathogenic microorganisms in foods. For example, in sliced and vacuum-packed cold smoked salmon, growth of *L. monocytogenes* has often been found to cease when lactic acid bacteria reach their maximum cell concentration. This so-called Jameson effect can be modelled by a simple expansion of the differential form of the logistic model (Ross et al., 2000; Dalgaard, 2002). In the baseline survey, however, no information on the populations of lactic acid bacteria on the tested samples was collected.

Various modelling approaches (see approaches 1 and 3 in Part II of the External Report) could be used to describe satisfactorily the data. Owing to the above limitations, however, these models would not have had the appropriate biological basis, and thus their application to a different set of data could lead to erroneous predictions.

An alternative approach to the development of predictive models for the growth of *L. monocytogenes* could be the validation of existing predictive models for *L. monocytogenes* against the data derived from the survey (see approach 2 in Part II of the External Report). Development of models to predict

growth of microorganisms in foods has been a particularly active research area within food microbiology during the last 25 years (Ross and Dalgaard, 2003). Among the various pathogens, *L. monocytogenes* is probably the one with the largest number of available growth models. Some of these models have been incorporated into user-friendly software tools. Today, a considerable number of predictive microbiology software tools are available, either as freeware or with restricted access, to predict survival/growth of microorganisms in foods, including *L. monocytogenes*. A process termed 'validation' allows the assessment of the reliability of models before they are used to aid decisions. This typically involves the comparison of model predictions with analogous observations not used to develop the model. Consequently, the validation procedure requires the input parameters of the models (both intrinsic and extrinsic factors of the particular food product under consideration in the validation study) to be known. However, as is stressed above, such information is unknown for several variables in the samples of the baseline survey. In addition, validation requires the estimation of the observed kinetic parameters in order to compare them with those predicted by the models. So the obstacles described for the development of the models remain also for the validation.

In conclusion, the limitations of the available information and of the nature and characteristics of the collected baseline survey data, as described above, did not allow the use of these data for the development of satisfactorily accurate predictive models for the microbial growth of *L. monocytogenes* under various storage conditions, using the classical predictive microbiology approach. For the same reasons, it would not have been useful to further compare analytically test results at time of sampling and at the end of shelf-life, in addition to presenting the *L. monocytogenes* counts in Figures 1, 2 and 3.

3.5. Predictive models for compliance with *Listeria monocytogenes* food safety criteria in foods

This ToR was addressed by developing and applying a statistical methodology which might allow a Competent Authority to extrapolate from the findings of a prevalence survey of RTE foods to assessing the concept of 'compliance' within that population of RTE foods.

Commission Regulation 2073/2005 mentions two microbiological criteria applicable for RTE foods at different stages. The criterion with which compliance might usefully be considered at the retail stage is the requirement for RTE foods not to harbour *L. monocytogenes* counts in excess of 100 cfu/g at the end of shelf-life. In addition to analysis of fish product samples at time of sampling, in the present study samples from all three surveyed RTE food categories were stored in the laboratory under refrigeration until the end of shelf-life before being tested. The fundamental requirement to predict compliance from this prevalence survey, therefore, involves consideration of what a survey comprising single-unit samples ($n = 1$) might represent for the surveyed population of RTE foods if a multiple-unit sample approach ($n = 5$) had been followed.

3.5.1. Statistical methodology

In statistical terms, the probability of compliance for this exercise was defined as the probability that no individual unit, out of $n = 5$ units constituting a sample taken from a population of RTE foods, exceeds the level of 100 cfu/g, at the end of shelf-life. The estimation of this probability is based on an estimate of the proportion of samples with *L. monocytogenes* counts exceeding the level of 100 cfu/g, obtained from a single-unit sample survey in the same population of RTE foods. The approach taken was to develop and apply a statistical model that would extrapolate from available data to compliance with a microbiological criterion requiring none of five sample units, for example, from one batch, to exceed 100 cfu/g at the end of shelf-life.

The statistical methodology followed was to use the beta-binomial distribution for clustered binary data and available data on the proportion of samples having an *L. monocytogenes* count > 100 cfu/g from a single-unit sample survey, in order to estimate the theoretical probability that if a five-unit sample had been taken from the batch from which the individual units originated all five out of five units, would have been compliant (i.e. having *L. monocytogenes* counts not exceeding 100 cfu/g).

A key component of this methodology is the consideration of the 'within-batch' correlation amongst the units in a sample from the same batch, and various possible values for this input were considered in developing and applying the model. In this report, the model focuses on a design where a sample includes units originating from the same batch but the statistical methodology could be used for any five-unit sample survey.

4. Results and discussion

Listeria monocytogenes is a facultative food-borne pathogen that colonises many habitats within and outside the food chain. *L. monocytogenes* might be vertically introduced to food premises through contaminated raw materials, or horizontally as a result of environmental contamination during food processing (Wiedmann, 2002). During processing, multiple technological barriers, such as heat treatment, acidification, drying or preservation, are applied to raw materials to limit outgrowth of *L. monocytogenes*. Listeriae are also considered as poor competitors against competing commensal flora (Carnio et al., 1999). However, initial raw product contamination may introduce *L. monocytogenes* in the production environment, and cross-contamination scenarios must be anticipated. In the event of insufficient Good Manufacturing Practice[8] (EFSA, 2005) it is possible that *L. monocytogenes* from unclean areas (where contaminated raw material is manipulated) will be transmitted to areas where, for example, heat-treated RTE product is manipulated. Such recontamination events could introduce *L. monocytogenes* to a pre-final or final product and facilitate growth because the contamination event is mainly targeting the food surface and the indigenous flora might have been reduced in previous steps of food processing. For some food categories, such as smeared soft cheeses, the present flora creates environments enabling *Listeria* to grow (Schoder et al., 2013). Subsequent storage of food, even if packed under modified atmosphere or vacuum packed, can prevent or limit the growth but is not likely to eliminate *L. monocytogenes* from the food product. A further factor adapting *L. monocytogenes* to storage conditions in modern food production is that *L. monocytogenes* is capable of growing at refrigeration temperatures (Gandhi and Chikindas, 2007). This physiological advantage over most other food-borne pathogens is even more important to consider when RTE food products are stored for extended periods of time.

In a number of cases, food products may be contaminated from the environment through hygiene failures (Stessl et al., 2014). This is why listeriosis has been addressed as a saprophytosis rather than a zoonoses in older literature. A crucial role in understanding the contamination dynamics is how *L. monocytogenes* survive in food processing environments (FPEs) despite being exposed to either physical stress or treatments by chemicals used as sanitisers. Abiotic vectors such as food contact materials may play a crucial role in *L. monocytogenes* transmission and contamination of conveyor belts and slicers has been reported to facilitate spread of *L. monocytogenes* into the food chain (Almeida et al., 2013). Special attention has been given in recent years to persistent colonisation of environmental niches within food business operations with *L. monocytogenes*. Whether persistence is only a passive effect due to poor sanitation or driven by selection of special *L. monocytogenes* clones signified by genetically encoded traits resulting in adaption is under debate (Carpentier and Cerf, 2011). Recent research has unravelled clones of *L. monocytogenes* that carry mobile elements partly explaining an increased survival capacity against sublethal concentrations of antagonistic chemical substances such as sanitisers (Elhanafi et al., 2010; Müller et al., 2013).

In conclusion, many yet scarcely understood factors enable *L. monocytogenes* to survive and multiply throughout the food chain. Food processing environments can be hotspots for spreading adapted clones. There is high variability in the probability of *L. monocytogenes* growth owing to food intrinsic, extrinsic and implicit factors, and extrapolating conclusions from one food chain to another is not feasible. A strength of the recently published prevalence estimates in the EFSA Report Part A (EFSA, 2013), originating from the EU-wide baseline survey, is the comprehensive view on the European situation by sampling more than 10 000 food items from three RTE food categories previously shown

[8] Good Manufacturing Practice covers the principles needed to design plant layout, equipment and procedures for the production of safe food. This includes hygienic operation and cleaning and disinfection procedures. The codes and requirements may be formally specified by e.g. Codex Alimentarius Committee on Food Hygiene.

to be at risk of contamination. Getting more insight into factors related to the prevalence of *L. monocytogenes* in these RTE food categories is desirable and is a core activity in this report, Part B.

In general, the true prevalence of *L. monocytogenes* in RTE food products depends on the contamination level of the raw material, the processing conditions (i.e. smoking conditions) and the opportunities for cross-contamination (i.e. during slicing). However, the estimated prevalence at retail is also indirectly affected by other factors which determine the growth of the pathogen during storage, such as the physicochemical characteristics of the product (i.e. pH, a_w, presence and concentration of antimicrobials), the packaging atmosphere, the storage temperature and the storage time. Indeed, for a contaminated product in which the above factors allow extensive growth during storage, the probability of detecting the pathogen at retail can be considerably higher.

According to the EU summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2012 (EFSA and ECDC, 2014), five strong-evidence food-borne outbreaks caused by *L. monocytogenes* were reported in the EU by three MSs, resulting in nine deaths. Mixed food (sandwiches), bakery products (pork pies), bovine meat and products thereof (pressed beef), cheese and other or mixed red meat and products thereof (meat jelly) were the implicated foods. In 2011, three strong-evidence food-borne outbreaks caused by *L. monocytogenes* were reported by the EU MSs; the food vehicles implicated were domestically produced cheese, bakery products and mixed food (EFSA and ECDC, 2013). In a Scientific Opinion of the Panel on Biological Hazards it was indicated that the prevalence and contamination levels of *L. monocytogenes* in different food types had been associated with factors including the 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 (Hazard Analysis and Critical Control Points) system, and lack of education and training of food handlers (EFSA, 2007b).

4.1. Results of GEE models for factors associated with the prevalence of *Listeria monocytogenes* and with the proportion of samples with a *Listeria monocytogenes* count > 100 cfu/g in surveyed food products

In this and following sections, the results are presented in the form of ORs with CIs and *P*-values, estimated each time for a specific category of a factor, compared with another category of the same factor. For continuous variables the OR refers to the comparison of the odds of the outcome in a sample with a one-unit higher value of the variable, compared with the odds for a sample with a one-unit lower value.

In the current analysis, the high number of factors affecting the modelled outcomes (prevalence and proportion of samples with an *L. monocytogenes* count > 100 cfu/g) in combination with the, frequently, very large variability in the characteristics of the food items and the great imbalance in the distribution of the food items among the levels of several factors, made the analysis difficult, owing to sparseness problems. This was greatly exacerbated by the small number of baseline survey samples that were positive for the examined outcomes, which led, on many occasions, to a very unequal distribution of the data among the categories defined by the combinations of the levels of all factors and the modelled outcomes in each model, including, on occasion, cells which contained no data at all. For example, a cross-classification of the variable indicating the presence of *L. monocytogenes* against the 'Type of retail outlet' original variable, while accounting for the 'EC 2073/2005 NSG' variable would clearly show the sparseness for the retail outlet types 'Speciality delis' and 'Street market/farmers' market', with categories with observed frequencies not exceeding 3, mostly being 0 or 1 (see External Report). As another example, it can be noted that the variable 'EC 2073/2005 NSG' had to be removed from the multiple-factor model for fish samples at the end of shelf-life, because it was preventing the model from converging because of sparseness, since no single sample included in 'EC 2073/2005 NSG' had an *L. monocytogenes* count above 100 cfu/g. These problems were evident during the model-building process and also 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 among 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 results, in this report, focuses mainly on the factors which were significantly associated with the modelled outcomes, exhibiting consistent and stable associations in the presented models and the corresponding sensitivity analyses.

The interaction terms between 'Packaging type' and 'Storage temperature at retail', as well as between 'Packaging type' and 'Storage temperature at laboratory up to the end of shelf-life' (together with the respective main effects) were initially included in the corresponding multiple-factor models both for fish and for meat product samples, since they were considered to be potentially biologically relevant, as explained above. However, these interaction terms were not significantly associated with the outcome in any of the multiple-factor models; therefore, they were not retained in the final models. The absence of a significant association with either prevalence or counts exceeding 100 cfu/g might be attributed to the low study power arising from data sparseness. However, it should be noted that, in addition to temperature, other factors, such as initial headspace concentration of CO_2, the gas to product ratio and the fat content of the product, impact on the antimicrobial effect of a CO_2-enriched atmosphere on *L. monocytogenes*' ability to grow (Devlieghere et al., 1998).

4.1.1. Analysis of factors related to the prevalence of *Listeria monocytogenes*-contaminated fish samples and to the proportion of fish samples with a *Listeria monocytogenes* count > 100 cfu/g, both at time of sampling and at the end of shelf-life

Four final models are presented: for *L. monocytogenes* prevalence in fish products at time of sampling and at the end of shelf-life; and for the proportion of samples with *L. monocytogenes* counts > 100 cfu/g in fish products at time of sampling and at the end of shelf-life. Table 1 shows the results of the multiple-factor analysis at both time-points in the case of *L. monocytogenes* prevalence. Table 2 shows the final model outcomes in the case of proportion of samples with *L. monocytogenes* counts > 100 cfu/g, at both testing time-points (time of sampling and end of shelf-life).

Concerning prevalence, the factors that exhibited mostly significant and consistent effects were 'Subtype of the fish product' (which is related to processing), 'Number of antimicrobial preservatives and/or acidity regulators' and 'Possible slicing'. Concerning the multiple-factor models for the proportion of samples with *L. monocytogenes* counts > 100 cfu/g, the factor that showed mostly significant and consistent association with the outcome was 'Possible slicing'.

Table 1: Results from the GEE models for prevalence of *L. monocytogenes* in packaged hot or cold smoked or gravad fish samples at time of sampling and at end of shelf-life, in the EU[(a)], 2010-2011

Factors related to prevalence of *L. monocytogenes* in fish samples				Time of sampling			End of shelf-life		
Variable	**Baseline category**	**Category compared with baseline category**	**Subset to which the OR applies**	**OR**	**95 % CI**	***P*-value**	**OR**	**95 % CI**	***P*-value**
Subtype of the fish product	Cold smoked fish	Gravad fish	All samples	0.72	0.45 – 1.16	0.18	0.86	0.53 – 1.40	0.55
		Hot smoked fish		0.54	0.33 – 0.89	0.02	0.61	0.38 – 0.98	0.04
		Unknown smoked fish		0.57	0.41 – 0.79	0.001	0.62	0.45 – 0.86	0.004
Number of antimicrobial preservatives and/or acidity regulators (AP/AR)	No reported AP/AR	One AP/AR	All samples	0.55	0.20 – 1.49	0.24	0.60	0.20 – 1.77	0.36
		Two or more AP/AR		7.89	4.33 – 14.39	< 0.0001	7.15	3.61 – 14.17	< 0.0001
Possible slicing	Not sliced	Sliced	All samples	1.59	1.02 – 2.48	0.04	1.39	0.91 – 2.12	0.13
Fish species	Salmon	Herring	For samples in the category 'Not included in EC 2073/2005 NSG'	1.09	0.56 – 2.12	0.81	1.05	0.55 – 2.01	0.88
			For samples in the category 'Included in EC 2073/2005 NSG'	0.84	0.14 – 4.93	0.85	0.27	0.03 – 2.75	0.27
Fish species	Salmon	Mackerel	For samples in the category 'Not included in EC 2073/2005 NSG'	0.52	0.29 – 0.93	0.03	0.33	0.17 – 0.64	0.001
			For samples in the category 'Included in EC 2073/2005 NSG'	3.51	0.97 – 12.75	0.06	2.37	0.65 – 8.71	0.19
Fish species	Salmon	Mixed fish	For samples in the category 'Not included in EC 2073/2005 NSG'	0.46	0.27 – 0.79	0.01	0.65	0.40 – 1.06	0.08
			For samples in the category 'Included in EC 2073/2005 NSG'	0.65	0.13 – 3.12	0.59	0.31	0.03 – 2.88	0.30
Fish species	Salmon	Other fish	For samples in the category 'Not included in EC 2073/2005 NSG'	0.80	0.48 – 1.35	0.41	0.80	0.48 – 1.34	0.40
			For samples in the category 'Included in EC 2073/2005 NSG '	0.56	0.10 – 3.12	0.51	0.12	0.01 – 1.65	0.11

Table continued overleaf.

Table 1: Results from the GEE models for prevalence of *L. monocytogenes* in packaged hot or cold smoked or gravad fish samples at time of sampling and at end of shelf-life, in the EU[(a)], 2010-2011 (continued)

Factors related to prevalence of *L. monocytogenes* in fish samples				Time of sampling			End of shelf-life		
Variable	**Baseline category**	**Category compared with baseline category**	**Subset to which the OR applies**	**OR**	**95 % CI**	***P*-value**	**OR**	**95 % CI**	***P*-value**
Storage temperature at laboratory up to the end of shelf-life	Storage at a temperature	Storage at 1 °C higher	For samples in the category 'Not included in EC 2073/2005 NSG'	N/A			0.91	0.82 – 1.02	0.10
			For samples in the category 'Included in EC 2073/2005 NSG'				1.68	1.03 – 2.73	0.04
Sampling season	Winter	Autumn	All samples	Not in final model			1.76	1.23 – 2.52	0.002
		Spring					0.98	0.65 – 1.47	0.91
		Summer					1.39	0.95 – 2.03	0.09
EC 2073/2005 NSG	Not included in EC 2073/2005 NSG	Included in EC 2073/2005 NSG	For 'Salmon'	0.52	0.21 – 1.33	0.17	0.75[9]	0.34 – 1.65	0.47
			For 'Herring'	0.41	0.08 – 2.11	0.29	0.19	0.02 – 1.87	0.16
			For 'Mackerel'	3.56	1.41 – 9.00	0.007	5.42	1.50 – 19.58	0.01
			For 'Mixed fish'	0.74	0.19 – 2.89	0.67	0.35	0.04 – 2.90	0.33
			For 'Other fish'	0.37	0.08 – 1.69	0.20	0.11	0.01 – 1.55	0.10

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this analysis.

[9] EC 2073/2005 NSG: in the multiple-factor model for samples tested at the end of shelf-life this variable participated in two significant interaction terms—one with 'Fish species' and one with 'Storage temperature at laboratory up to the end of shelf-life'. For this reason, the presentation of the results for variable 'EC 2073/2005 NSG' needs to also consider these other two variables. Therefore, results describing the effect of 'EC 2073/2005 NSG' on *L. monocytogenes* prevalence are presented separately for each fish species, but also it has to be noted that the presented ORs for each fish species have been calculated for a given storage temperature (4.22 °C, the mean 'Storage temperature at laboratory up to the end of shelf-life' of the dataset). These ORs would have been different if they were calculated for any other specific storage temperature. Consequently, interpretation of these ORs should be made with caution and only with reference to the specific storage temperature.

Table 2: Results from the GEE models for proportion of packaged hot or cold smoked or gravad fish samples with an *L. monocytogenes* count exceeding the level of 100 cfu/g at sampling and at end of shelf-life, in the EU[(a)], 2010-2011

Factors related to proportion of fish samples with *L. monocytogenes* count > 100 cfu/g				Time of sampling			End of shelf-life		
Variable	**Baseline category**	**Category compared with baseline category**	**Subset to which the OR applies**	**OR**	**95 % CI**	***P*-value**	**OR**	**95 % CI**	***P*-value**
Type of retail outlet (modified variable)	Supermarket or small shop	All other types of retail outlet	All samples	Not in final model			4.29	1.29 – 14.22	0.02
Sampling season	Winter	Autumn	All samples	1.35	0.34 – 5.44	0.67	1.39	0.64 – 3.0	0.41
		Spring		1.65	0.39 – 6.92	0.49	0.28	0.08 – 1.02	0.05
		Summer		4.29	1.22 – 15.03	0.02	1.45	0.66 – 3.21	0.36
Possible slicing	Not sliced	Sliced	All samples	2.79	0.90 – 8.58	0.07	2.55	1.07 – 6.05	0.03
EC 2073/2005 NSG	Not included in EC 2073/2005 NSG	Included in EC 2073/2005 NSG	All samples	0.55	0.08 – 3.94	0.55	Not in final model		

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this analysis.

4.1.1.1. Subtype of the fish product

This variable reflected the different types of processing that the fish products had undergone. With respect to samples tested at the time-point of sampling, the OR of being contaminated with *L. monocytogenes* for 'Hot smoked fish' compared with 'Cold smoked fish' was 0.54 (significantly lower than 1; *P*-value = 0.02). Consequently, the odds of a sample being contaminated with *L. monocytogenes* were significantly lower for 'Hot smoked fish' than for 'Cold smoked fish'. The OR for being *L. monocytogenes*-positive for 'Unknown smoked fish' (fish which may have been hot or cold smoked) compared with 'Cold smoked fish' was 0.57 (*P*-value = 0.001). The odds of being contaminated with *L. monocytogenes* for 'Gravad fish' compared with 'Cold smoked fish' were not significantly different (*P*-value = 0.18). However, in sensitivity analysis using weighting, the OR for that comparison became statistically significant.

Regarding the samples that were tested at the end of shelf-life, the association of *L. monocytogenes* prevalence with 'Subtype of the fish product' was remarkably similar to that at time of sampling. The odds of being contaminated with *L. monocytogenes* for a sample of 'Hot smoked fish' or of 'Unknown smoked fish' were significantly lower than for 'Cold smoked fish'. Again, the odds of *L. monocytogenes* contamination of 'Gravad fish' and 'Cold smoked fish' were not significantly different.

Concerning the multiple-factor models for the proportion of samples with an *L. monocytogenes* count > 100 cfu/g, the variable 'Subtype of the fish product' was not included in either of the models (for time of sampling and for the end of shelf-life), as there was no significant association between this variable and the outcome.

Smoking is traditionally applied as a preservation method; it has the potential to destroy bacteria (bactericidal effects) and inhibits subsequent growth (bacteriostatic effects). Smoking transforms raw fish into an RTE product, prolongs product durability, and gives particular flavour and organoleptic characteristics. Smoking can imply cold smoking (temperatures below 30 °C) or hot smoking (temperatures > 60 °C). The preservation effect of smoking results from various compounds, including formaldehydes and phenols; moreover, together with salting and drying, smoking results in lower a_w in the product and formation of a protective, more membranous surface (Rørvik, 2000). Gravad fish production is a preservation method which involves pickling in an acid environment to produce an RTE product.

In the present study a key finding was that 'Cold smoked fish' had almost twice the odds of being contaminated with *L. monocytogenes* than either 'Hot smoked fish' or 'Unknown smoked fish', both in samples tested at the time of sampling as well as in those tested at the end of shelf-life. This is in agreement with published literature; Jørgensen and Huss (1998) found that *L. monocytogenes* prevalence was highest in cold smoked fish (34–60 %), and lowest in heat-treated seafood, including hot smoked fish (4–12 %). Although processing temperatures used in the smoked fish industry can vary, the higher temperatures applied during the hot smoking process could at least partially explain the lower odds of *L. monocytogenes* contamination in 'Hot smoked fish' compared with 'Cold smoked fish'. The listericidal effect of hot smoking has been demonstrated, and depends both on temperature and smoke type (Jemmi and Keusch 1992; Poysky et al., 1997). In contrast, cold smoking cannot completely destroy *L. monocytogenes* because the applied temperatures are not sufficiently high (Eklund et al., 1995; Rørvik, 2000; Sabanadesan et al., 2000).

Gravad fish production also lacks a thermal treatment step, which could explain the observed lack of difference shown in prevalence in the present study when compared with 'Cold smoked fish'. Data on *L. monocytogenes* prevalence in gravad fish are quite limited (Loncarevic et al., 1996; Jørgensen and Huss, 1998), though gravad fish are products that have also been associated with risk of human listeriosis (Loncarevic et al., 1998).

Owing to the unknown smoking status of the fish samples included in the 'Unknown smoked fish' category, it is difficult to interpret the lower odds of *L. monocytogenes* contamination of the 'Unknown smoked fish' compared with 'Cold smoked fish'. The fact that 'Unknown smoked fish' was, by far, the most frequently reported category, accounting for more than half of the total fish samples taken from retail, may indicate a need for better labelling of smoked fish. The unknown smoking status of a large proportion of the fish samples is probably a drawback to this analysis, in the sense that clearer and/or stronger associations between 'Subtype of the fish product' and *L. monocytogenes* prevalence might have been observed if the type of smoking was specified for a higher proportion of the smoked fish samples.

4.1.1.2. Number of antimicrobial preservatives and/or acidity regulators (AP/AR)

The OR of being contaminated with *L. monocytogenes* for samples with 'Two or more AP/AR' was over seven times that of samples with 'No reported AP/AR', both at the time of sampling and at the end of shelf-life (Table 1). This association was found to be statistically significant in both cases, and it was quite stable across additional modifications of the models (i.e. according to the results of sensitivity analyses). Conversely, whilst not significant, samples with 'One AP/AR' showed lower odds of being contaminated with *L. monocytogenes* than samples with 'No reported AP/AR' both at time of sampling and at the end of shelf-life. Furthermore, no significant association with the 'Number of antimicrobial preservatives and/or acidity regulators (AP/AR)' emerged in the models for the proportion of samples with *L. monocytogenes* counts > 100 cfu/g (Table 2).

Initial appraisal of this association, i.e. the higher odds of *L. monocytogenes* contamination in fish samples with 'Two or more AP/AR' than in fish with 'No reported AP/AR', might appear something of a paradox, given that the addition of antimicrobial preservatives and/or acidity regulators in processed fish, as well as in other food categories, is practised in order to inhibit or at least delay bacterial growth and thereby extend the spoilage- and/or food safety-driven shelf-life of foods. 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). Furthermore, any related conclusions or even attempts to interpret the association between the 'Number of antimicrobial preservatives and/or acidity regulators (AP/AR)' and *L. monocytogenes* prevalence should be made with great caution because the number of reported additives in this baseline survey 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.

Tables 3 and 4 provide some further insights into the actual data of the reported 'Number of antimicrobial preservatives and/or acidity regulators (AP/AR)' for samples tested at time of sampling (considerations are similar for the data for samples tested at the end of shelf-life). In trying to explain this apparent paradox of higher numbers of additives being associated with higher odds of *L. monocytogenes* presence, one could hypothesise that (the majority of) these samples may have originated from a limited number of manufacturers that might have processed/manufactured these products under lower hygiene standards. However, this does not seem to be the case, since the 55 samples with 'Two or more AP/AR' were obtained from 9 different countries, 25 cities and 45 retail outlets. Another hypothesis would be that these 55 samples had longer intended shelf-lives and, consequently, the manufacturers of these products might have tended to add more preservatives in order to stabilise them, especially products that are more conducive to growth of *L. monocytogenes*. However, the paucity of production date data, and, hence, shelf-life data, did not allow the testing of this hypothesis. Finally, one can only speculate that the (majority of) these products might have originated from manufacturers who, having had a history of frequent *L. monocytogenes*-positive test

results as part of their own checks or competent authority testing, tended to rely heavier on the use of food antimicrobials as a means of inhibiting *L. monocytogenes* growth during product shelf-life.

Table 3: Cross-classification table between contamination of *L. monocytogenes* in packaged hot or cold smoked or gravad fish samples, at time of sampling and the 'Number of antimicrobial preservatives and/or acidity regulators (AP/AR)' in the EU[(a)], 2010-2011

Number of antimicrobial preservatives and/or acidity regulators (AP/AR)	Total	Contaminated	%
No reported AP/AR	2 915	284	9.74
One AP/AR	83	4	4.82
Two or more AP/AR	55	25	45.45
Total	3 053	313	10.25

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.

Table 4: Cross-classification table between contamination of *L. monocytogenes* in packaged hot or cold smoked or gravad fish samples, further separated by 'EC 2073/2005 NSG' at time of sampling, and the 'Number of antimicrobial preservatives and/or acidity regulators (AP/AR)' in the EU[(a)], 2010-2011

Number of antimicrobial preservatives and/or acidity regulators (AP/AR)	Not included in EC 2073/2005 NSG			Included in EC 2073/2005 NSG		
	Total	Contaminated	%	Total	Contaminated	%
No reported AP/AR	2 749	271	9.86	166	13	7.83
One AP/AR	54	3	5.56	29	1	3.45
Two or more AP/AR	40	20	50	15	5	33.33
Total	2 843	294	10.34	210	19	9.05

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.

4.1.1.3. Possible slicing

At the time of sampling, the odds of 'Sliced' fish being contaminated with *L. monocytogenes* were 1.59 times the odds in 'Not sliced' fish (*P*-value = 0.04). While the odds of contamination in 'Sliced' products remained higher than in 'Not sliced' products at the end of shelf-life, that result was not statistically significant (*P*-value = 0.13). The OR of having an *L. monocytogenes* count above 100 cfu/g for a 'Sliced' sample compared with a 'Not sliced' sample was 2.79 (*P*-value = 0.07) at the time of sampling and 2.55 (*P*-value = 0.03) at the end of shelf-life. In addition to this consistent and frequently significant association across the four models, this finding appeared to be robust in sensitivity analysis using weighted analysis.

Sliced smoked fish products are generally produced by slicing of whole smoked sides, i.e. the slicing is a handling process that arises after the most listericidal step in the process. Therefore, whilst the residual phenolic compounds of smoke may exert an ongoing bacteriostatic effect, the process of slicing creates additional potential for post-process contamination (Jemmi and Keusch, 1992, 1994; Rørvik et al., 1995; Autio et al., 1999; Huss et al., 2000; Rørvik, 2000; Dass et al., 2010). Slicing entails additional handling steps by food workers in food processing plants and slicing can serve as a source of contamination with bacteria originating from personnel, the packaging material, but most

importantly from slicing equipment such as knives and blades (Vogel et al., 2001). Slicing machines are one of the most difficult types of equipment to clean and decontaminate in the food industry (Aarnisalo et al., 2006, 2007), and have been repeatedly identified as a source of *L. monocytogenes* contamination (Autio et al., 1999; Miettinen et al., 2001).

4.1.1.4. Fish species and EC 2073/2005 NSG

The models indicated significant interaction terms between the variables 'EC 2073/2005 NSG' and 'Fish species' for the outcome of *L. monocytogenes* prevalence[10], but not for the outcome of proportion of samples with counts exceeding 100 cfu/g (in which 'Fish species' does not appear as a factor at all). There was a general trend in both prevalence models for similar or lower (usually non-significantly different) odds of contamination for most fish species when compared with 'Salmon'. For 'Mackerel' samples compared with 'Salmon' samples, the prevalence models showed that samples in the category 'Not included in EC 2073/2005 NSG' had ORs significantly lower than 1, meaning that the odds of being contaminated with *L. monocytogenes* were lower in these 'Mackerel' samples in the category 'Not included in EC 2073/2005 NSG' than in 'Salmon' samples in the same category. However, conversely, for samples in the category 'Included in EC 2073/2005 NSG', the odds of 'Mackerel' being contaminated compared with the odds for 'Salmon' were higher at both sampling times, with a tendency towards significance at the time of sampling (*P*-value = 0.06).

As regards the variable 'EC 2073/2005 NSG', the ORs comparing the odds of being contaminated with *L. monocytogenes* for samples in the category 'Included in EC 2073/2005 NSG' with those in the category 'Not included in EC 2073/2005 NSG' were lower than 1 for all 'Fish species' categories, except for 'Mackerel'. In the case of 'Mackerel' samples, the OR of *L. monocytogenes* contamination was significantly higher than 1 for samples in the category 'Included in EC 2073/2005 NSG', compared with samples in the category 'Not included in EC 2073/2005 NSG', both at the time of sampling and at the end of shelf-life. Appraisal of the actual data, seen in Table 5 for samples tested at time of sampling (considerations are similar for the data for samples tested at the end of shelf-life), demonstrates that these apparently counter-intuitive associations with 'EC 2073/2005 NSG' status for 'Mackerel' arise from a small number of 'Mackerel' samples in the category 'Included in EC 2073/2005 NSG' (41 of 3 053) with a remarkably high proportion of contaminated samples (7 out of 41 or 17.1 %). Prudence in interpretation is, therefore, necessary, although there was no particular evidence of clustering in the spread of the 24 contaminated 'Mackerel' samples (17 in the category 'Not included in EC 2073/2005 NSG' and 7 in the category 'Included in EC 2073/2005 NSG'), which were obtained from 7 countries, 16 cities and 16 outlets.

Table 5: Cross-classification table between 'Fish species', contamination with *L. monocytogenes* and 'EC 2073/2005 NSG' variable in packaged hot or cold smoked or gravad fish samples, at time of sampling, in the EU[(a)], 2010-2011

Fish species	Not included in EC 2073/2005 NSG			Included in EC 2073/2005 NSG		
	Total	Contaminated	%	Total	Contaminated	%
Herring	143	15	10.49	40	2	5.0
Mackerel	369	17	4.61	41	7	17.07
Mixed fish	304	18	5.92	22	3	13.64
Other fish	234	21	8.97	41	2	4.88
Salmon	1 793	223	12.44	66	5	7.58
Total	2 843	294	10.34	210	19	9.05

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.

[10] In the multiple-factor model for samples tested at the end of shelf-life the variable 'EC 2073/2005 NSG' participated in two significant interaction terms—one with 'Fish species' and one with 'Storage temperature at laboratory up to the end of shelf-life'. Therefore, the ORs describing the effect of 'EC 2073/2005 NSG' on *L. monocytogenes* prevalence are presented separately for each fish species and have been calculated for a given storage temperature (4.22 °C).

In conclusion, the apparent 'Fish species' effect appears to be a reflection of an association with 'EC 2073/2005 NSG' arising from a small number (7) of contaminated samples in 'Mackerel' in the category 'Included in EC 2073/2005 NSG'. Moreover, as the corresponding model results were not always stable in sensitivity analysis, these findings should not be over-interpreted.

Finally, no significant association between 'EC 2073/2005 NSG' status and the proportion of samples with *L. monocytogenes* counts exceeding 100 cfu/g at either time-point was found in the multiple-factor analysis. In fact, the variable 'EC 2073/2005 NSG' had to be removed from the multiple-factor model for fish samples at the end of shelf-life, because it was preventing the model from converging owing to sparseness, since no single sample that was in the category 'Included in EC 2073/2005 NSG' had an *L. monocytogenes* count exceeding 100 cfu/g.

4.1.1.5. Storage temperature at laboratory up to the end of shelf-life

This temperature variable had a significant effect (i.e. the OR of being contaminated with *L. monocytogenes* at a given storage temperature compared with a storage temperature 1 °C lower was significantly different from 1) only for samples in the category 'Included in EC 2073/2005 NSG' (OR = 1.68, *P*-value = 0.04). This finding is rather counter-intuitive; however, it did not always survive sensitivity analysis and, therefore, should be interpreted with caution. This variable was also not included in the models investigating factors related to the proportion of samples with *L. monocytogenes* counts > 100 cfu/g.

4.1.1.6. Sampling season

The 'Sampling season' variable was significantly associated with *L. monocytogenes* prevalence (for samples tested at the end of shelf-life) as well as with the outcome concerning samples having an *L. monocytogenes* count exceeding 100 cfu/g, at both sampling times. However, the 'Sampling season' is unlikely to be a particularly accurate indicator of the production season and associations are not easily interpretable and may require further investigation. Moreover, these results were not always stable during sensitivity analysis with use of weighting, and should be interpreted with caution.

4.1.1.7. Type of retail outlet

The OR of having an *L. monocytogenes* count above 100 cfu/g for a sample that had been obtained from 'All other types of retail outlet' (as defined in Section 3.3.2.4) compared with a sample that had been obtained from a 'Supermarket or small shop' was 4.29 (*P*-value = 0.02). However, in sensitivity analysis (exact logistic regression model including also the variable 'EC 2073/2005 NSG'), the OR for the 'Type of retail outlet' variable was not significant (*P*-value = 0.10). Moreover, the number of samples belonging to the 'All other types of retail outlets' category was rather small (49 out of 3 053), and the majority of these samples (44 out of 49) originated from outlet types that were originally classified under 'Other' in terms of their 'Type of retail outlet' status. Therefore, this finding should not be over-interpreted.

4.1.2. Analysis of factors related to the prevalence of *Listeria monocytogenes*-contaminated meat product samples and the proportion of meat product samples with a count of *Listeria monocytogenes* > 100 cfu/g at the end of shelf-life

Two final models are presented: for *L. monocytogenes* prevalence and for the proportion of samples with *L. monocytogenes* counts > 100 cfu/g in meat products at the end of shelf-life. Tables 6 and 7 list the final results from these two models. Concerning prevalence, the factors that exhibited mostly significant and consistent effects were 'Type of the meat product' and 'Possible slicing'. Concerning the multiple-factor models for the proportion of samples with *L. monocytogenes* counts exceeding 100 cfu/g, the factors that showed the most consistently significant association with the outcome were 'Animal species of the origin of the meat product' and 'Remaining shelf-life'.

4.1.2.1. Type of the meat product:

The OR of a 'Pâté' sample being contaminated with *L. monocytogenes* compared to 'Cold, cooked meat product' was 2.91 (*P*-value = 0.005). However, the odds of being contaminated with *L. monocytogenes* for 'Sausage' samples were not statistically significantly different from the respective odds for 'Cold, cooked meat product' (OR = 0.97, *P*-value = 0.93).

All meat products sampled should have been subjected to a heat treatment step during processing, sufficient for inactivation of any occasional *L. monocytogenes* present in the raw materials. Moreover, all meat products had to be handled after heat treatment leading to a possibility for recontamination of the product. In comparison to samples in the categories 'Cold, cooked meat product' and 'Sausage', the production of products in the category 'Pâté' generally involves more post-cooking handling. 'Pâté' products may also be covered with gelatine and/or be decorated with other foodstuffs. In those cases, the dressing process and the products used may serve as additional sources of *L. monocytogenes* contamination.

Finally, it is interesting to note that even though the variable 'Type of the meat product' was significantly associated with *L. monocytogenes* prevalence in meat product samples, this variable was not included in the final model for the proportion of packaged heat-treated meat product samples with an *L. monocytogenes* count exceeding the level of 100 cfu/g.

Table 6: Results from the GEE model for prevalence of *L. monocytogenes* in packaged heat-treated meat product samples, at the end of shelf-life, in the EU[(a)], 2010-2011

Factors related to prevalence of *L. monocytogenes* in packaged heat-treated meat products				**End of shelf-life**		
Variable	**Baseline category**	**Category compared with baseline category**	**Subset to which the OR applies**	**OR**	**95 % CI**	***P*-value**
Type of the meat product	Cold, cooked meat product	Pâté	All samples	2.91	1.39 – 6.10	0.005
		Sausage		0.97	0.52 – 1.82	0.93
Possible slicing	Not sliced	Sliced	All samples	2.13	0.94 – 4.83	0.07
Packaging type (modified variable)	All other packaging types	Modified atmosphere	All samples	0.60	0.36 - 0.99	0.048

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this analysis.

Table 7: Results from the GEE model for proportion of packaged heat-treated meat product samples with an *L. monocytogenes* count exceeding the level of 100 cfu/g, at the end of shelf-life, in the EU[(a)], 2010-2011

Factors related to proportion of meat samples with *L. monocytogenes* count > 100 cfu/g				**End of shelf-life**		
Variable	**Baseline category**	**Category compared with baseline category**	**Subset to which the OR applies**	**OR**	**95 % CI**	***P*-value**
Animal species of the origin of the meat product	Avian species	All other species	All samples	0.35	0.13 – 0.97	0.04
Possible Slicing	Not sliced	Sliced	All samples	2.61	0.33 – 20.53	0.36
Remaining Shelf-life	Number of days of remaining shelf-life	One additional day of remaining shelf-life	All samples	1.010	1.005 – 1.016	0.0002

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this analysis.

4.1.2.2. Possible slicing

The odds of a sample being contaminated with *L. monocytogenes* for 'Sliced' meat products were 2.13 times the odds for 'Not sliced' meat products with a *P*-value of 0.07. Moreover, it was noted that 'Possible slicing' was statistically significantly associated with *L. monocytogenes* contamination in the analysis using weights (OR = 2.63, *P*-value = 0.03, when using weights based on planned sample sizes and OR = 2.92, *P*-value = 0.01, when using weights based on the size of the population in each country). The odds of 'Sliced' meat products having an *L. monocytogenes* count above 100 cfu/g were 2.61 times the odds for 'Not sliced' meat products but the difference was not significant (*P*-value = 0.36).

Slicing is an additional handling step post heat treatment and may function as an additional source of contamination with *L. monocytogenes*. As outlined before, for the fish products sampled in the present study, slicing machines have been identified as a source of *L. monocytogenes* contamination also for cold cooked meat products, (Uyttendaele et al., 1999; Gillespie et al., 2000; FSAI, 2011). In the Uyttendaele et al. (1999) study the set of cooked meat products made from whole muscles (e.g. cooked ham, cooked loin, cooked poultry breast) sampled as intact meat products showed a lower prevalence of *L. monocytogenes* than the set of sliced meat products (1.56 % and 6.65 %, respectively).

4.1.2.3. Animal species of the origin of the meat product

The OR of a meat product sample having an *L. monocytogenes* count above 100 cfu/g for 'All other species' compared with 'Avian species' was 0.35 (*P*-value = 0.04), indicating that the odds were significantly lower for products originating from 'All other species' of animals than for products originating from 'Avian species'. The OR associated with the 'Animal species of the origin of the meat product' was non-significant (*P*-value = 0.06) in the weighted analyses using weights based on the planned sample sizes (however, in the model fitted with the method of Firth the OR was significantly different from 1 in both weighted analyses). Literature data indicate that many more broiler carcasses are contaminated with *L. monocytogenes* than pig and beef carcasses (Autio et al., 2000; Rørvik et al., 2003; Lindblad et al., 2006, 2007; Wieczorek et al., 2012; Khen et al., 2014). Handling of raw meat which is more frequently contaminated may lead to a higher risk of contamination of the production area, where heat-treated products are further handled, leading to recontamination of the products.

4.1.2.4. Packaging type

The odds of a sample being contaminated with *L. monocytogenes* for products packaged under 'Modified atmosphere' were significantly lower than the corresponding odds for samples with 'All other packaging types' (OR = 0.60, *P*-value = 0.048). As mentioned previously, the presence of increased concentrations of CO_2 in the modified packaging atmosphere may impact on the growth potential of *L. monocytogenes* and thus, indirectly, on the probability of detection of *L. monocytogenes*, while also the water solubility of CO_2 increases if the temperature drops, thus enhancing the antimicrobial effect (Devlieghere et al., 2001). However, as also mentioned before, in addition to temperature, other factors impact on the antimicrobial effect of a CO_2-enriched atmosphere on *L. monocytogenes*' ability to grow (Devlieghere et al., 1998). In the present analysis, the effect of 'Packaging type' was not stable, as it was not significant in the weighted analysis (OR = 0.63, *P*-value = 0.10, when using weights based on planned sample sizes and OR=0.61, *P*-value = 0.09, when using weights based on the size of the population in each country). Consequently, one should be careful with formulating strong statements about the effect of this factor. Moreover, in the present study, no significant association of 'Packaging type' with the proportion of meat products with *L. monocytogenes* counts exceeding 100 cfu/g was noted.

4.1.2.5. Remaining shelf-life:

The OR of a meat product sample having an *L. monocytogenes* count above 100 cfu/g compared with a sample whose 'Remaining shelf-life' is one day shorter was 1.010 (95 % CI: 1.005–1.016: statistically significantly higher than 1, for a level of significance equal to 0.05). This seems to indicate increased odds of having an *L. monocytogenes* count above 100 cfu/g as the 'Remaining shelf-life' increases, within the ranges observed in the dataset. The interpretation of this finding is not straightforward and any strong statements about the effect of this factor should be avoided. It should be noted that the sampling time was an arbitrary point during the shelf-life of the product and the actual length of the shelf-life was not known for most of the products in the baseline survey. It is possible that the variable 'Remaining shelf-life' can be regarded as an indirect indicator of the actual shelf-life of the product. In this case, a longer remaining shelf-life would tend to indicate a product with a longer overall shelf-life, which could point to a more stable product, microbiologically. However, a longer remaining shelf-life may lead, in a number of cases, to the outgrowth of *L. monocytogenes* to a level above 100 cfu/g. It is also interesting to note that this variable was not included in any of the final models for the fish samples, or in the model for prevalence of *L. monocytogenes* in the packaged heat-treated meat product samples.

Finally, it is also interesting to note that in the present study the variable 'Storage temperature at laboratory up to the end of shelf-life' was not included in any of the two models, for prevalence and for proportion of samples with a count of *L. monocytogenes* exceeding 100 cfu/g, for meat product samples.

4.1.3. Caution in the interpretation of and conclusions from statistical models

More advanced statistical models and methods aim to account for all complexities in the data, as far as possible. The results from the final multiple-factor models, however, have to be interpreted with caution, for several reasons:

- Statistically significant effects point at numerical associations which are unlikely to have arisen purely due to chance, but which cannot be interpreted necessarily as causal relationships, whereby the presence of a factor could be seen to somehow cause the outcome found.
- In the current analysis, the large number of factors affecting the modelled outcomes (prevalence and proportion of samples with an *L. monocytogenes* count > 100 cfu/g) in combination with the, frequently, very large variability in the characteristics of the food items and the great imbalance in the distribution of the food items among the levels of several factors, made the analysis difficult, owing to sparseness problems. It can be expected that many factors and explanatory variables may cause heterogeneity in the prevalence. However, owing to the high dimensionality and the sparseness of the data in particular combinations, it is not possible to take all sources of heterogeneity into account. As known and discussed extensively in the literature (see, for example, Agresti, 2013), this implies that one has to be careful with interpretation of estimated effects. To get some further insights into the impact of the sparseness on the stability of the statistical model findings, the final models were refitted with exact logistic regression and with Firth's method, as part of the sensitivity analyses.
- As discussed above, the sampling size did not perfectly follow the survey design, and the survey design did not perfectly reflect population sizes. For that reason, unweighted analyses were complemented with weighted analyses with different types of weights, but all weights were proxy weights for the unknown true weights. Therefore, the weighted analyses were considered as part of the sensitivity analyses, but, again, the estimated effects should not be over-interpreted.

Nevertheless, despite the above considerations, the data and the results from the fitted multiple-factor models can provide new insights, by confirming the role of certain factors as known from the literature, or by pointing at some unexpected effects, which then can be examined in more detail. Such

investigations might shed some light on the complex interplay of several factors on the prevalence of *L. monocytogenes* and on the proportion of samples with an *L. monocytogenes* count that exceeded the level of 100 cfu/g, in the surveyed RTE foods.

4.2. Predictive models for compliance with *Listeria monocytogenes* food safety criteria in foods

4.2.1. Development of the statistical model

As mentioned in Section 3.5.1 the purpose of the statistical model is to use available data on the proportion of samples having an *L. monocytogenes* count > 100 cfu/g from a single-unit sample survey, in order to estimate the theoretical probability that if a five-unit sample had been taken from the batch from which the individual units originated, all five out of five units, would have been compliant (i.e. having *L. monocytogenes* counts not exceeding 100 cfu/g).

The model developed was based on the beta-binomial distribution (see, for example, Aerts et al., 2002) for clustered binary data. The model can be described as follows:

n is the number of units constituting the sample.

π is the probability of a random unit from a random sample (e.g. from a batch) having an *L. monocytogenes* count that exceeds the level of 100 cfu/g.

ρ is the 'within-batch' correlation for samples with $n > 1$, i.e. any two randomly selected units originating from the same sample from a batch might be correlated in their *L. monocytogenes* count being above 100 cfu/g. This expression of correlation would, therefore, describe the tendency of any two units in a sample from a batch either to have or not have, simultaneously, an *L. monocytogenes* count above 100 cfu/g. In the extreme case that $\rho = 1$, all units in a sample from the same batch would have values above 100 cfu/g, or none of them would. If $\rho = 0$, the event of one unit in a sample from the batch having a count above 100 cfu/g would be independent of the event of any other unit in the sample having a count that exceeds the value of 100 cfu/g.

In case of a single unit ($n = 1$), the probability of compliance depends directly on the probability π through the formula

$$C(\pi) = 1 - \pi$$

If two or more units are tested ($n > 1$) within a sample from the same batch, compliance depends not only on the probability π, but also on the within-batch correlation ρ. A well-established probability model for clustered binary data is the beta-binomial model, extending the binomial model for independent units to correlated units (see, for example, Aerts et al., 2002). Using the beta-binomial probability model, the probability of compliance can be modelled as:

$$\mathrm{C}(\pi, \rho) = \mathrm{P}(\text{compliance}) = \mathrm{P}\left(0 \text{ out of } n \text{ exceed } 100\frac{\text{cfu}}{\text{g}}\right)$$

$$C(\pi, \rho) = (1 - \pi)\left(1 - \pi + \frac{\pi\rho}{1}\right)\left(1 - \pi + \frac{2\pi\rho}{1+\rho}\right)\ldots\left(1 - \pi + \frac{(n-1)\pi\rho}{1+(n-2)\rho}\right)$$

If the sample units are independent ($\rho = 0$), then the previous equation becomes:

$$C(\pi, 0) = (1 - \pi)^n$$

If the sample units are perfectly correlated (ρ = 1) then:

$$C(\pi, 1) = 1 - \pi$$

The function $C(\pi, \rho)$ is flexible enough to allow for the estimation of the compliance probability for any particular case (given a point estimate or CI for its input parameters π and ρ). If ρ is known, an estimate $\hat{\pi}$ for proportion π and a 95% CI $(\hat{\pi}_l, \hat{\pi}_u)$ can be used to derive a point estimate $C(\hat{\pi}, \rho)$ and a 95% CI $(C(\hat{\pi}_u, \rho), C(\hat{\pi}_l, \rho))$ for the compliance probability. If one has knowledge about a range of plausible values for ρ, say the interval $(\rho_{\min}, \rho_{\max})$, and using the monotonicity of $C(\pi, \rho)$ as a function of ρ, one can take the uncertainty about ρ into account by taking the CI: $(C(\hat{\pi}_u, \rho_{\min}), C(\hat{\pi}_l, \rho_{\max}))$. In the scenario that there is absolutely no knowledge of ρ, one can use the CI: $(C(\hat{\pi}_u, 0), C(\hat{\pi}_l, 1))$. Further details are given in the External Report.

The resulting estimate of the probability of compliance is affected by the point estimate $\hat{\pi}$ and also by the inputted degree of correlation among the *n* units sampled from the batch. Reliable estimates for this parameter were not observable from the present dataset; therefore, an assumption would be necessary. For example, units may be very highly correlated if they all originate from the same batch and all sampled food items within this batch have the exact same outcome. If it is not possible to make an informed assumption about ρ, then a conservative approach can be used, in which the possible range of values for ρ is considered to be between 0 and 1.

4.2.2. Application of the statistical model

Estimates for the proportion of samples with *L. monocytogenes* counts exceeding the level of 100 cfu/g for several RTE foods were obtained in the EFSA Scientific Report, Part A (EFSA, 2013). The statistical model developed above was then used to estimate the probability of compliance (as defined previously), based upon the survey data. The methodology provided a spread of likely compliance outcomes depending on the presumption made concerning the variation amongst sample subunits, i.e. the different values inputted for the correlation parameter. The results presented in this section do not consider the data from Norway.

Table 8 shows the point estimate and 95 % CI for the probability of compliance for a range of within-batch correlation values, for fish, meat and cheese samples from the baseline survey, at the end of shelf-life. The last row of the table for each RTE food type corresponds to the 'conservative approach', in which the possible range of values for ρ is considered to be able to take any value between 0 and 1. Figures 4, 5 and 6 show plots of the point estimate and the corresponding 95 % CI (upper and lower limit) for the probability of compliance as a function of the within-batch correlation ρ (ranging from 0 to 1) for fish, meat and cheese samples, at the end of shelf-life, respectively. The red vertical line represents the 'conservative approach' CI, using the minimum and maximum limits of all CIs (for values of ρ from 0 to 1) for that food item.

Table 8: Point estimate and 95 % CI for the probability of compliance for a range of within-batch correlation values, for RTE food products sampled for the *L. monocytogenes* baseline survey, in the EU[(a)], 2010-2011. $\hat{\pi}$, $\hat{\pi}_l, \hat{\pi}_u$ are the prevalence estimates from the survey (point estimate, lower and upper limit of the 95 % CI) and ρ is the value of the within-batch correlation. The last row of the table for each RTE food type corresponds to the 'conservative approach', in which the confidence interval is composed by the minimum and maximum limits of all confidence intervals for the corresponding RTE food type

Fish product samples at end of shelf-life $\hat{\pi} = 1.7$ % $(\hat{\pi}_l, \hat{\pi}_u) = (1.3$ %, 2.3 %)		
ρ	$C(\hat{\pi}, \rho)$	$(C(\hat{\pi}_u, \rho), C(\hat{\pi}_l, \rho))$
0.0	0.918	(0.890,0.937)
0.1	0.931	(0.908,0.947)
0.5	0.962	(0.948,0.971)
0.9	0.979	(0.972,0.984)
1.0	0.983	(0.977,0.987)
–	(0.918,0.983)	(0.890,0.987)
Meat product samples at end of shelf-life $\hat{\pi} = 0.43$ % $(\hat{\pi}_l, \hat{\pi}_u) = (0.25$ %, 0.74 %)		
ρ	$C(\hat{\pi}, \rho)$	$(C(\hat{\pi}_u, \rho), C(\hat{\pi}_l, \rho))$
0.0	0.979	(0.964,0.987)
0.1	0.982	(0.970,0.990)
0.5	0.990	(0.983,0.994)
0.9	0.995	(0.991,0.997)
1.0	0.996	(0.993,0.997)
–	(0.979,0.996)	(0.964,0.997)
Cheese samples at end of shelf-life $\hat{\pi} = 0.059$ % $(\hat{\pi}_l, \hat{\pi}_u) = (0.015$ %, 0.236 %)		
ρ	$C(\hat{\pi}, \rho)$	$(C(\hat{\pi}_u, \rho), C(\hat{\pi}_l, \rho))$
0.0	0.997	(0.988,0.999)
0.1	0.998	(0.990,0.999)
0.5	0.999	(0.995,1.000)
0.9	0.999	(0.997,1.000)
1.0	0.999	(0.998,1.000)
–	(0.997,0.999)	(0.988,1.000)

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are not included in this analysis.

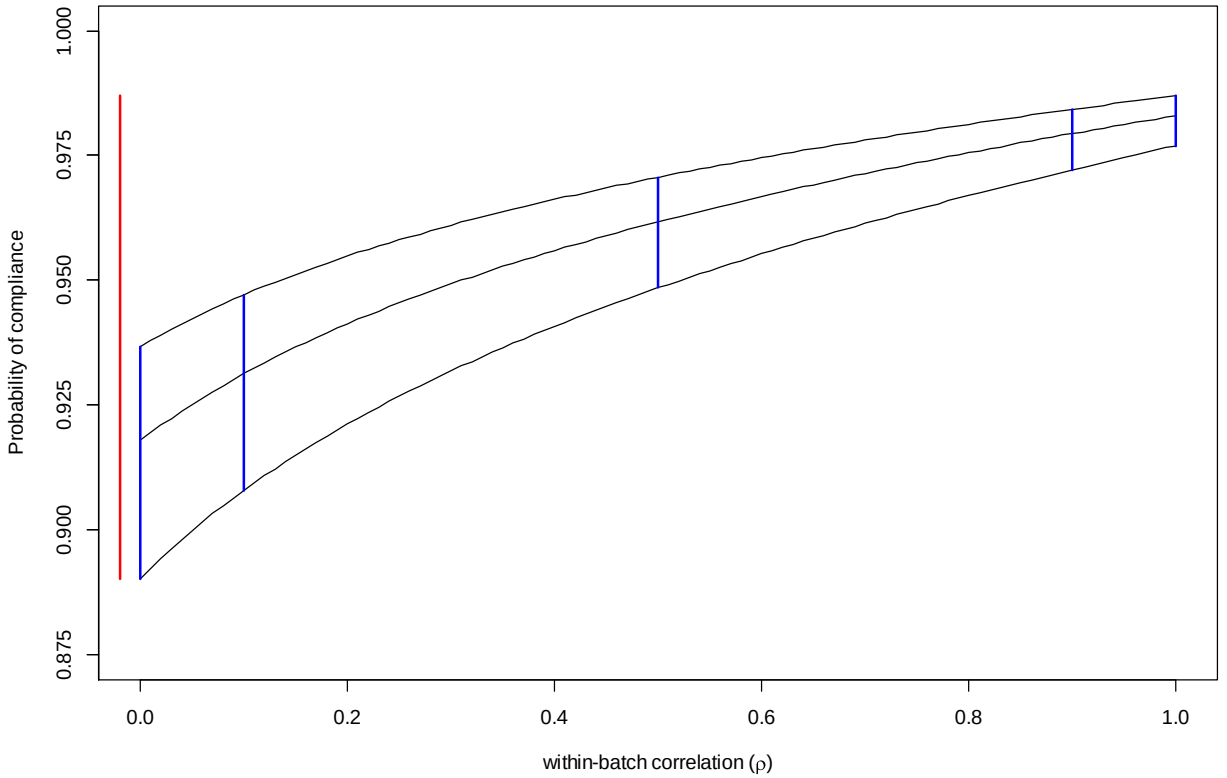

Figure 4: Point estimate and 95 % CI for the probability of compliance, based on prevalence estimates obtained from the EU-wide baseline survey, for fish product samples, at the end of shelf-life, in the EU, 2010-2011 (Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are not included in this analysis). The red vertical line represents the 'conservative approach' CI, using the minimum and maximum limits of all CIs (for values of ρ from 0 to 1).

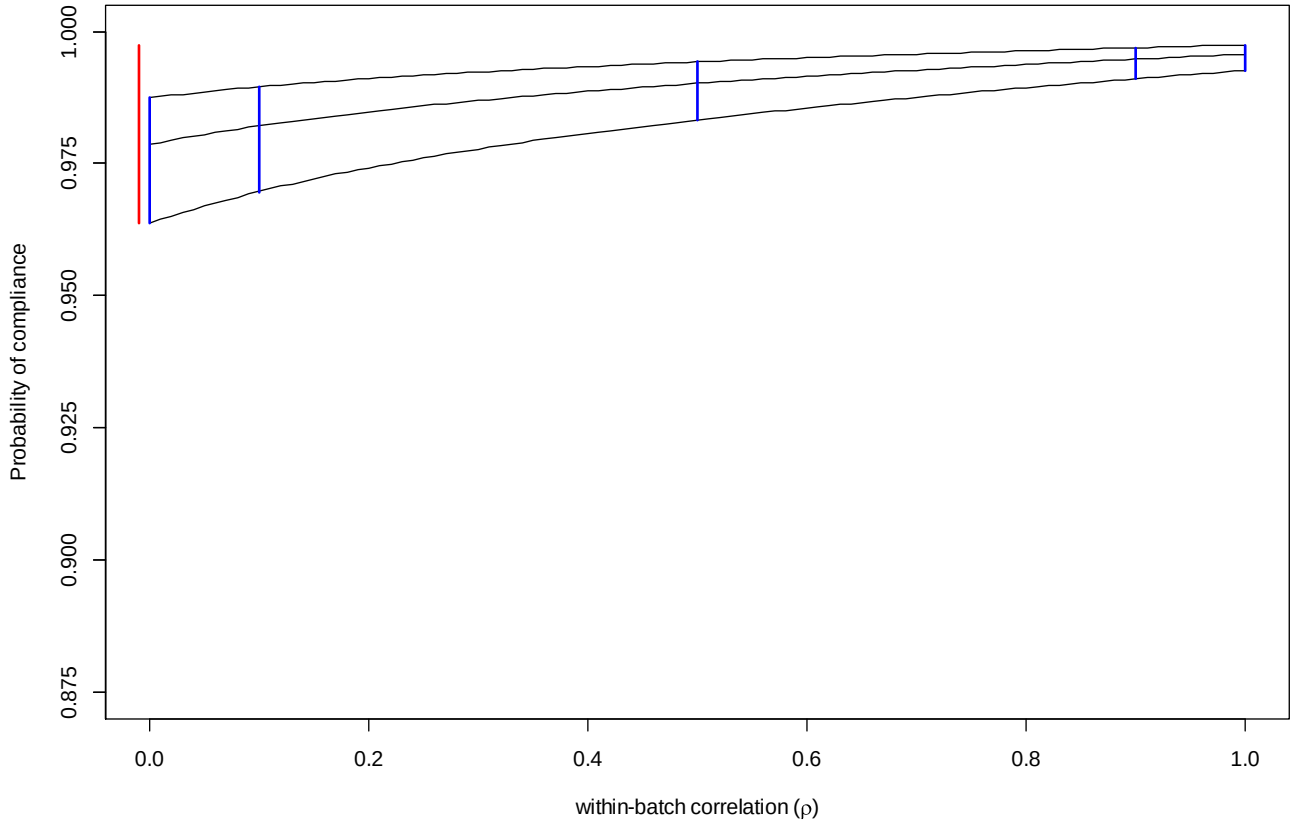

Figure 5: Point estimate and 95 % CI for the probability of compliance, based on prevalence estimates obtained from the EU-wide baseline survey, for meat product samples, at the end of shelf-life, in the EU, 2010-2011 (Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are not included in this analysis). The red vertical line represents the 'conservative approach' CI, using the minimum and maximum limits of all CIs (for values of ρ from 0 to 1).

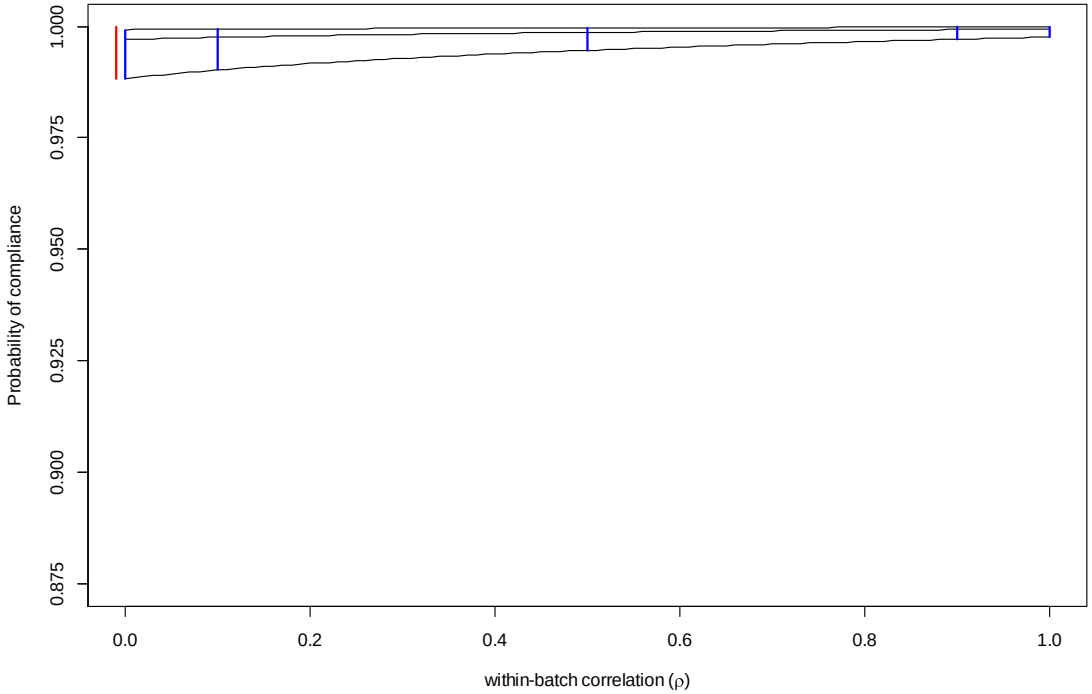

Figure 6: Point estimate and 95 % CI for the probability of compliance, based on prevalence estimates obtained from the EU-wide baseline survey, for cheese samples, at the end of shelf-life, in the EU, 2010-2011 (Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are not included in this analysis). The red vertical line represents the 'conservative approach' CI, using the minimum and maximum limits of all CIs (for values of ρ from 0 to 1).

4.2.3. Discussion of the statistical model developed and applied

The statistical model selected was a relatively simple approach, essentially based upon a beta-binomial model. This model may provide some basis by which a single-unit sample survey could be used to consider the probability of compliance. Lower point prevalence estimates would intuitively result in higher modelled compliance probability, as can be seen when comparing fish with meat or cheese samples. The estimated compliance probability would be less than simply 100 % minus the point prevalence estimate, except when the within-batch correlation is 1. This is because any one of five sample units with an *L. monocytogenes* count exceeding 100 cfu/g is sufficient to regard the entire sample as non-compliant. Thus, testing of units from a five-unit sample is more likely to detect a non-compliant batch than testing of a one-unit sample.

A key input to this model, and therefore a key consideration in assessing the output, is the degree of relatedness amongst sample units within a sample from a batch, with respect to their *L. monocytogenes* count being above 100 cfu/g. From extreme perspectives, this input could be considered as no relatedness whatsoever, so one unit in a sample from the batch having a count above 100 cfu/g would be independent of the event of any other unit in the sample having a count exceeding 100 cfu/g; or absolute relatedness, whereby all units in a sample from the same batch would have *L. monocytogenes* counts above 100 cfu/g, or none of them would.

In the present study, there was no information available on whether some samples from the baseline survey could be originating from the same batch, so no data were available to estimate the degree of correlation with any degree of certainty. Considering, at a theoretical level, the notion of relatedness of units within a sample from a batch, the very concept of individual units originating from a larger batch would suggest a degree of correlation greater than zero, but the biological reality of heterogeneity of bacterial distribution within a batch would tend to a degree of correlation less than 1. Moreover, the definition of batch in Commission Regulation 2073/2005 has no size or time limitations, so the degree of correlation might well have varied quite substantially across the, potentially, thousands of batches analysed.

Various different values of the correlation parameter ρ were, therefore, considered for the present report. In all cases, it can be seen that for a given π if there is an assumption of high within-batch correlation then the derived estimates for the probability of compliance are higher and the CIs become narrower. For example, regarding fish at the end of shelf-life making an assumption that units are independent ($\rho = 0$), the probability of compliance is estimated to be 0.918 (95 % CI: 0.890 – 0.937). However, if the five units are assumed to be perfectly correlated, the estimated probability of compliance increases to 0.983 (95 % CI: 0.977–0.987). If no information is available on the correlation, the 'conservative approach' could be followed, meaning that ρ would be allowed to take any value between 0 and 1 and the point estimate for the probability of compliance would be between 0.918 and 0.983. In this case, a CI could be constructed using the minimum and maximum limits of all CIs for all possible values of the correlation parameter ρ, i.e. 0.89 to 0.987.

In order to obtain the best estimate of the probability of compliance with an appropriately narrow CI, the input required to the model regarding the degree of correlation should be actively considered. Such consideration would be informed by assessment of available information including food product homogeneity, batch size, or analysis of five-unit sample datasets in analogous products. A homogenised liquid batch of food might well be expected to have a high degree of correlation amongst sample units. However, other food production scenarios might justify a lower correlation value. For example, a production system involving a large number of discrete production units (e.g. salmon sides) might produce units that do not cluster so tightly, with respect to their probability of having an *L. monocytogenes* count above 100 cfu/g. In the absence of available information, a conservative approach would be to consider a range of values for within-batch correlation between 0 and 1, with resultant range of derived compliance probabilities.

The strength of the methodology applied in the present study is its relative simplicity. The weakness is the reliance on information regarding the within-batch correlation, which is frequently lacking. Overall, such a model would allow maximum value to be extrapolated from available official control analysis sampling, and some understanding of overall compliance to be derived from single-unit sampling approaches.

This potential utility of such a statistical method would not alter the obligation on food business operators, which explicitly remains in Commission Regulation 2073/2005, to analyse $n = 5$ samples, in order to demonstrate compliance. The statistical methodology, developed and applied in this report, should not be seen as a way to facilitate demonstration of compliance by food business operators using fewer than five sample units. This method may have some utility when, for example, a Competent Authority has carried out a prevalence survey in a population of RTE foods, based upon a representative sampling plan, and wishes to make some assessment of compliance within that population of RTE foods.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

This Part B report provides results from further analysis of the baseline survey on the prevalence of *Listeria monocytogenes* in certain RTE foods in the EU. This was the first baseline survey directly investigating foodstuffs at retail, and it was also the first baseline survey enabling the estimation of the prevalence only at the EU level and not at MS level. An important characteristic of this survey that greatly affected the statistical analyses is that, even though a large number of samples were obtained during the baseline survey, the variety of the obtained samples was very large and the number of *L. monocytogenes*-contaminated samples and the number of samples with counts exceeding 100 cfu/g were small. Concerning the presented multiple-factor modelling, it should be emphasized that the statistical associations revealed by the models between various factors and the modelled outcomes do not necessarily constitute causal relations.

- The number of factors affecting the modelled outcomes (prevalence and proportion of samples with an *L. monocytogenes* count that exceeded the level of 100 cfu/g) in the two categories of RTE foods (smoked and gravad fish and heat-treated meat products) can be high. This is partly because the closer to the consumer level that analysis of an RTE food occurs, the more factors there are that may affect the studied outcomes because the food has undergone more interventions, e.g. transportation and storage for variable periods of time at variable, and often unknown, temperatures.

- The baseline survey was designed so that reliable EU-level estimates for *L. monocytogenes* prevalence in three RTE food categories could be obtained. Using the survey's data for multiple-factor analysis created issues of sparseness, owing to the large number of factors examined and the small number of surveyed RTE food samples that were *L. monocytogenes* positive or that had an *L. monocytogenes* count exceeding 100 cfu/g. This resulted in a very low or even zero number of samples in specific sample sub-categories, which made the analysis difficult. The large variety in RTE food products and manufacturing technology, even within an apparently well-defined food category, exacerbated this issue of sparseness.

- Problems due to sparseness were evident during the model-building process and also 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 among 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 results, in this report, focuses mainly on the

factors which were significantly associated with the modelled outcomes, exhibiting consistent and stable associations in the presented models and the corresponding sensitivity analyses.

- As regards the factors associated with *Listeria monocytogenes* prevalence in the packaged (not frozen) hot or cold smoked or gravad fish samples, the odds of *Listeria monocytogenes* presence were higher for 'Cold smoked fish' than for 'Hot smoked fish' and 'Unknown smoked fish', for 'Sliced' than for 'Not sliced' samples and for samples with 'Two or more antimicrobial preservatives and/or acidity regulators', than for samples with 'No reported antimicrobial preservatives and/or acidity regulators'.

- For packaged (not frozen) hot or cold smoked or gravad fish samples, the proportion of samples with *Listeria monocytogenes* counts exceeding 100 cfu/g was associated with 'Possible slicing'; 'Sliced' fish samples had higher odds of containing *L. monocytogenes* in excess of 100 cfu/g than 'Not sliced' samples.

- As regards the factors associated with *Listeria monocytogenes* prevalence in the packaged heat-treated meat products, higher odds of *Listeria monocytogenes* presence were found for 'Pâté' than for 'Cold, cooked meat products' and for 'Sliced' samples than for 'Not sliced' samples.

- For packaged heat-treated meat products, the proportion of samples with *Listeria monocytogenes* counts exceeding 100 cfu/g was associated with the 'Animal species of the origin of the meat product' (higher odds for products made from meat from 'Avian species') and with 'Remaining shelf-life' (the OR of having an *L. monocytogenes* count above 100 cfu/g was slightly higher for a meat product sample with a given 'Remaining shelf-life' than for a sample with a 'Remaining shelf-life' that was one day shorter).

- After extensive analysis of the available eligible data from pairs of fish product samples for the development of predictive models, it was concluded that, given the limitations of the available information and of the nature and characteristics of the collected baseline survey data, these data were not appropriate for the development of satisfactorily accurate predictive models for the growth of *L. monocytogenes* under various storage conditions.

- An understanding of likely probability of compliance with a microbiological criterion requiring that no individual unit out of $n = 5$ units constituting a sample exceeds 100 cfu/g can be derived from data obtained based on a single-unit sampling plan after necessary assumptions regarding the relatedness among the units within the sample. Concerning the model for compliance, the developed methodology may be used in situations where a prevalence survey has been carried out in a defined population of food items based upon a representative sampling plan and some assessment of compliance is desired for that population of foods.

RECOMMENDATIONS

- When a study for analysis of factors related to presence of a pathogen in a food population is desired, in situations in which the expected prevalence of a food-borne pathogen in specific food categories is low, some additional considerations might need to be given to the variability of the sampled products and the effective sample size per examined factor, in order to minimise the issues of sparseness that were evident in the current analysis.

- Food business operators producing cold smoked fish, pâté or sliced ready-to-eat smoked or gravad fish and heat-treated meat products might actively reconsider food safety management systems and their ongoing verification, in particular with increased attention to environmental *L. monocytogenes* sampling in the area of the slicing process, in order to ensure effective control of *L. monocytogenes* in their products.

- The presented statistical methodology that was developed as an effort to assist in the consideration of the concept of 'compliance' within a population of RTE foods may have some utility when, for example, a Competent Authority has carried out a prevalence survey in a population of RTE foods, based upon a representative sampling plan, and wishes to make some assessment of compliance within that population of RTE foods. However, this statistical methodology could not, and should not, be used as a substitute for the representativeness and legal obligation of a five-unit sampling approach which explicitly remains in the Commission Regulation 2073/2005 in order to demonstrate compliance.

REFERENCES

Aarnisalo K, Tallavaara K, Wirtanen G, Maijala R and Raaska L, 2006. The hygienic working practices of maintenance personnel and equipment hygiene in the Finnish food industry. Food Control, 17, 1001-1011.

Aarnisalo K, Sheen S, Raaska L and Tamplin M, 2007. Modelling transfer of *Listeria monocytogenes* during slicing of 'gravad' salmon. International Journal of Food Microbiology, 118, 69-78.

Aerts M, Geys H, Molenberghs G and Ryan L, 2002. Topics in modelling of clustered data. Chapman & Hall, London, UK, 336 pp.

Agresti A, 2013. Categorical Data Analysis. Wiley, Hoboken, New Jersey, 744 pp.

Almeida G, Magalhães R, Carneiro L, Santos I, Silva J, Ferreira V, Hogg T and Teixeira P, 2013. Foci of contamination of *Listeria monocytogenes* in different cheese processing plants. International Journal of Food Microbiology, 167, 303-309.

Autio T, Hielm S, Miettinen M, Sjöberg A-M, Aarnisalo K, Björkroth J, Mattila-Sandholm T and Korkeala H, 1999. Sources of *Listeria monocytogenes* Contamination in a Cold-Smoked Rainbow Trout Processing Plant Detected by Pulsed-Field Gel Electrophoresis Typing. Applied and Environmental Microbiology, 65, 150-155.

Autio T, Säteri T, Fredriksson-Ahomaa M, Rahkio M, Lundén J and Korkeala H, 2000. *Listeria monocytogenes* Contamination Pattern in Pig Slaughterhouses. Journal of Food Protection, 63, 1438-1442.

Carpentier B and Cerf O, 2011. Review—Persistence of *Listeria monocytogenes* in food industry equipment and premises. International Journal of Food Microbiology, 145, 1-8.

Carnio MC, Eppert I and Scherer S, 1999. Analysis of the bacterial surface ripening flora of German and French smeared cheeses with respect to their anti-listerial potential. International Journal of Food Microbiology, 47, 89-97.

Dalgaard P, 2002. Modelling and predicting the shelf-life of seafood. In: Safety and quality issues in fish processing. Ed. Bremner HA. Woodhead Publishing Ltd, Cambridge, UK, 191-219.

Dass SC, Abu-Ghannam N, Antony-Babu S and Cummins EJ, 2010. Ecology and molecular typing of *L. monocytogenes* in a processing plant for cold-smoked salmon in the Republic of Ireland. Food Research International, 43, 1529–1536.

Devlieghere F, Debevere J and Van Impe J, 1998. Concentration of carbon dioxide in the water-phase as a parameter to model the effect of a modified atmosphere on microorganisms. International Journal of Food Microbiology, 43 , 105-113.

Devlieghere F, Geeraerd AH, Versyck KJ, Vandewaetere B, Van Impe J and Debevere J, 2001. Growth of *Listeria monocytogenes* in modified atmosphere packed cooked meat products: a predictive model. Food Microbiology, 18, 53-66.

EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Panel on Biological Hazards on Campylobacter in animals and foodstuffs. The EFSA Journal 2005, 173, 1-10.

EFSA (European Food Safety Authority), 2007a. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2006, The EFSA Journal 2007, 130, 1-352.

EFSA (European Food Safety Authority), 2007b. Scientific Opinion of the Panel on Biological Hazards on a request from the European Commission on Request for updating the former SCVPH opinion on *Listeria monocytogenes* risk related to ready-to-eat foods and scientific advice on different levels of *Listeria monocytogenes* in ready-to-eat foods and the related risk for human illness. The EFSA Journal, 599, 1-42.

EFSA (European Food Safety Authority), 2009a. Report of Task Force on Zoonoses Data Collection on proposed technical specifications for a survey on *Listeria monocytogenes* in selected categories of ready-to-eat food at retail in the EU. The EFSA Journal 2009, 300, 1–66.

EFSA (European Food Safety Authority), 2009b. The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union in 2007. The EFSA Journal 2009, 223, 1-313.

EFSA (European Food Safety Authority), 2013. Analysis of the baseline survey on the prevalence of *Listeria monocytogenes* in certain ready-to-eat (RTE) foods in the EU, 2010-2011 Part A: *Listeria monocytogenes* prevalence estimates. EFSA Journal 2013;11(6):3241, 75 pp. doi:10.2903/j.efsa.2013.3241

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2013. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2011. EFSA Journal 2013;11(4):3129, 250 pp. doi:10.2903/j.efsa.2013.3129

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2014. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2012. EFSA Journal 2014;12(2):3547, 312 pp. doi:10.2903/j.efsa.2014.3547

Eklund MW, Poysky FT, Paranjpye RN, Lashbrook LC, Peterson ME and Pelroy GA, 1995. Incidence and Sources of *Listeria monocytogenes* in Cold-Smoked Fishery Products and Processing Plants. Journal of Food Protection, 58, 502–508.

Elhanafi D, Dutta V and Kathariou S, 2010. Genetic characterization of plasmid-associated benzalkonium chloride resistance determinants in a *Listeria monocytogenes* strain from the 1998-1999 outbreak. Applied and Environmental Microbiology, 76, 8231-8238.

Firth D, 1993. Bias reduction of maximum likelihood estimates. Biometrika, 80, 27-38.

FSAI (Food Safety Authority of Ireland), 2011. Establishing Baseline Data on the Presence of *Listeria monocytogenes* on Cooked Meat Slicers in Retail and Catering Premises. Available at: http://www.fsai.ie/establishingbaselinedataonthepresenceoflisteriamonocytogenesoncookedmeatslicersinretailandcateringpremises.html

Gandhi M and Chikindas ML, 2007. *Listeria*: A foodborne pathogen that knows how to survive. International Journal of Food Microbiology, 113, 1-15.

Gillespie I, Little C and Mitchell R, 2000. Microbiological examination of cold ready-to-eat sliced meats from catering establishments in the United Kingdom. Journal of Applied Microbiology, 88, 467–474.

Hirji KF, Mehta CR and Patel NR, 1987. Computing distributions for exact logistic regression. Journal of the American Statistical Association, 82, 1110-1117.

Huss HH, Jørgensen LV and Vogel BF, 2000. Control options for *Listeria monocytogenes* in seafoods. International Journal of Food Microbiology, 62, 267-274.

Jemmi T and Keusch A, 1992. Behavior of *Listeria monocytogenes* during processing and storage of experimentally contaminated hot-smoked trout. International Journal of Food Microbiology, 15, 339-346.

Jemmi T and Keusch A, 1994. Occurrence of *Listeria monocytogenes* in freshwater fish farms and fish-smoking plants. Food Microbiology, 11, 309-316.

Jørgensen LV and Huss HH, 1998. Prevalence and growth of *Listeria monocytogenes* in naturally contaminated seafood. International Journal of Food Microbiology, 42, 127-131.

Khen BK, Lynch OA, Carroll J, McDowell DA and Duffy G, 2014. Occurrence, Antibiotic Resistance and Molecular Characterization of *Listeria monocytogenes* in the Beef Chain in the Republic of Ireland. Zoonoses and Public Health, doi: 10.1111/zph.12106

Liang KY and Zeger SL, 1986. Longitudinal data analysis using generalized linear models. Biometrika, 73, 13-22.

Lindblad M, Lindmark H, Thisted Lambetz S and Lindqvist R, 2006. Microbiological baseline study of broiler chickens at Swedish slaughterhouses. Journal of Food Protection, 69, 2875-2882.

Lindblad M, Lindmark H, Thisted Lambertz S and Lindqvist R, 2007. Microbiological baseline study of swine carcasses at Swedish slaughterhouses. Journal of Food Protection, 70, 1790-1797.

Loncarevic S, Tham W and Danielsson-Tham ML, 1996. Prevalence of *Listeria monocytogenes* and other *Listeria* spp. in smoked and 'gravad' fish. Acta Veterinaria Scandinavica, 37, 8-13.

Loncarevic S, Danielsson-Tham M-L, Gerner-Smidt P, Sahlström L and Tham W, 1998. Potential sources of human listeriosis in Sweden. Food Microbiology, 15, 65-69.

Miettinen H, Aarnisalo K, Salo S and Sjöberg A-M, 2001. Evaluation of Surface Contamination and the Presence of *Listeria monocytogenes* in Fish Processing Factories. Journal of Food Protection, 64, 635-639.

Müller A, Rychli K, Muhterem-Uyar M, Zaiser A, Stessl B, Guinane CM, Cotter PD, Wagner M and Schmitz-Esser S, 2013. Tn6188—a novel transposon in *Listeria monocytogenes* responsible for tolerance to benzalkonium chloride. PLOS One, 8, e76835. doi:10.1371/journal.pone.0076835

Poysky FT, Paranjpye RN, Peterson ME, Pelroy GA, Guttman AE and Eklund MW, 1997. Inactivation of *Listeria monocytogenes* on Hot-smoked Salmon by the Interaction of Heat and Smoke or Liquid Smoke. Journal of Food Protection, 60, 649-654.

Rakhmawati TW, Nysen R and Aerts M, 2014. Statistical analysis of the *Listeria Monocytogenes* EU-wide baseline survey in certain ready-to-eat foods Part B*:* analysis of factors related to the prevalence of *Listeria Monocytogenes*, predictive models for the microbial growth and for compliance with food safety criteria. EFSA supporting publication 2014: EN-606, 368 pp.

Rørvik LM, 2000. *Listeria monocytogenes* in the smoked salmon industry. International Journal of Food Microbiology, 62, 183-190.

Rørvik LM, Caugant DA and Yndestad M, 1995. Contamination pattern of *Listeria monocytogenes* and other *Listeria* spp. in a salmon slaughterhouse and smoked salmon processing plant. International Journal of Food Microbiology, 25, 19-27.

Rørvik LM, Aase B, Alvestad T and Caugant DA, 2003. Molecular epidemiological survey of *Listeria monocytogenes* in broilers and poultry products. Journal of Applied Microbiology, 94, 633–640.

Ross T and Dalgaard P, 2003. Secondary models. In: Modeling microbial responses in food. Eds. McKellar RC and Lu X. CRC Press, Boca Raton, FL, USA, 63–150.

Ross T, Dalgaard P and Tienungoon S, 2000. Predictive modelling of the growth and survival of *Listeria* in fishery products. International Journal of Food Microbiology, 62, 231-245.

Sabanadesan S, Lammerding AM and Griffiths MW, 2000. Survival of *Listeria innocua* in Salmon following Cold-Smoke Application. Journal of Food Protection, 63, 715-720.

Schoder D, Skandamis P and Wagner M, 2013. Assessing in-house monitoring efficiency by tracing contamination rates in cheese lots recalled during an outbreak of listeriosis in Austria. International Journal of Food Microbiology, 167, 353–358.

Stessl B, Fricker M, Fox E, Karpiskova R, Demnerova K, Jordan K, Ehling-Schulz M and Wagner M, 2014. Collaborative Survey on the Colonization of Different Types of Cheese-Processing Facilities with *Listeria monocytogenes*. Foodborne Pathogens and Disease, 11, 8-14.

Tienungoon S, Ratkowsky DA, McMeekin TA and Ross T, 2000. Growth Limits of *Listeria monocytogenes* as a Function of Temperature, pH, NaCl, and Lactic Acid. Applied and Environmental Microbiology, 66, 4979-4987.

Uyttendaele M, De Troy P and Debevere J, 1999. Incidence of *Listeria monocytogenes* in different types of meat products on the Belgian retail market. International Journal of Food Microbiology, 53, 75–80.

Vogel BF, Huss HH, Ojeniyi B, Ahrens P and Gram L, 2001. Elucidation of *Listeria monocytogenes* contamination routes in cold-smoked salmon processing plants detected by DNA-based typing methods. Applied and Environmental Microbiology, 67, 2586-2595.

Wieczorek K, Dmowska K and Osek J, 2012. Prevalence, Characterization, and Antimicrobial Resistance of *Listeria monocytogenes* Isolates from Bovine Hides and Carcasses. Applied and Environmental Microbiology, 78, 2043-2045.

Wiedmann M, 2002. Molecular subtyping methods for *Listeria monocytogenes*. Journal of AOAC International, 85, 524-531.

APPENDICES

Appendix A. Data dictionary for coordinated monitoring programme for *Listeria monocytogenes* in certain ready-to-eat food categories at retail

European Commission DG Health and Consumers
Coordinated monitoring programme for *Listeria monocytogenes* in certain ready-to-eat food categories at retail

Version 5 dated 21 July 2010

All wording should be in English, as far as possible, in order of ease interpretation of the information

Block 1: Information on the place the sample was taken

Item Integer	Variable	Constraint	Definition	Description and Particularity	Type	Values
001	Country	Mandatory	Country in which the sampling has occurred	Must only be one of the values from the list or reference given in the 'Values' column	List element	ISO 3166-1-Alpha-2. All Member States + Norway, Iceland and Switzerland
002	Code of the town	Mandatory	Code of the town where the sample was taken	MS can define what they consider to be a town in the framework of this survey on the basis of their local knowledge of the geographical distribution of the population. It must be guaranteed that each town where samples have been taken in a country has a unique code throughout the survey. If more than one sample is taken in a town, the same code must be used. Postcodes are examples of values for this item.	Text	Alphanumeric
003	Code of retail outlet	Mandatory	Code of the outlet where the sample was taken	It must be guaranteed that each code of an outlet is unique within the same code of the town. If more than one sample is taken in the outlet, the same code must be used.	Text	Alphanumeric
004	Type of retail outlet	Mandatory	Type of retail outlet where the sample was taken	A supermarket or small shop is defined as a retail selling both food and non-food products. Speciality delis are shops selling high quality foods, such as special cheeses and cold cooked meat.	List element	(Supermarket or small shop); (Street market/farmers' market); (Speciality delis); (Other – freetype here)
005	Date of sampling	Mandatory	Date of collection of the sample	Date must not be < 15 December 2009 and not be > 15 January 2012	Date	ISO 8601 (YYYY-MM-DD)

Table continued overleaf.

Appendix A (continued). Data dictionary for coordinated monitoring programme for *Listeria monocytogenes* in certain ready-to-eat food categories at retail

Block 1: Information on the place the sample was taken						
Item Integer	**Variable**	**Constraint**	**Definition**	**Description and Particularity**	**Type**	**Values**
006	Type of sample	Mandatory	Type of RTE food that was sampled	Type of RTE food that was sampled	List element(s)	(Soft/semi-soft cheese); (Smoked or gravad fish); (Heat treated meat product)
007	Reference of the sample	Mandatory	Identifier of each RTE food sample	Sample must be uniquely identified. It must be guaranteed that at least the combination of this item 007 with item 002 (code of town), 003(code of retail outlet) and item 005 (date of sampling) is unique throughout the whole baseline survey. In the case of cheese and meat products there is only one sample from a batch. In the case of fishery products, two samples per batch will be collected but the information for the two samples will be submitted under a single unique sample reference. The complete information for the two fishery products samples will be submitted simultaneously after obtaining the results of testing at the end of shelf-life. Values for the common items 025 to 032 and 015 to 017 will be shared by both samples as these belong to the same batch.	Text	Alphanumeric
008	Comment	Optional	Any comment	MS can put additional information relevant to any specific point, in particular if clarification is needed when using "other" as value for certain items	Text	Alphanumeric values

Table continued overleaf.

Appendix A (continued). Data dictionary for coordinated monitoring programme for *Listeria monocytogenes* in certain ready-to-eat food categories at retail

Block 2: Appears if item "006" is "soft/semi-soft cheese"						
Item Integer	**Variable**	**Constraint**	**Definition**	**Description and Particularity**	**Type**	**Values**
009	Subtype of the cheese	Mandatory	Subtype of RTE cheese that was sampled	Subtype of RTE food that was sampled	List element	(Smear-ripened); (Mould-ripened); (Brine-matured); (Otherwise ripened); (unknown)
010	Type of milk treatment	Mandatory	Type of milk treatment as indicated on package	Type of milk treatment as indicated on package	List element	(Raw milk); (Thermised milk); (Pasteurized milk); (Unknown)
011	Animal of origin of the milk	Mandatory	Origin of milk used as indicated on package	Origin of milk used as indicated on package	List element	(Cow); (Sheep); (Goat); (Buffalo); (Mixed); (Unknown)
012	Packaging place for cheese	Mandatory	Packaging conditions of the RTE cheese selected for sampling	Was the RTE cheese packaged by original producer, packaging centre or at retail?	List element	(Packaged by the producer or re-packed at packaging centre); (Re-packed at retail) (Unknown)
013	Cheese rind included in the analysis	Mandatory			Boolean	Yes No
014	Percentage of rind	Optional	Appears only if answer to item 013 is "yes"	Estimated percentage of rind	List element	< = 20 % Between 20 % and 40 % > = 40 %

Table continued overleaf.

Appendix A (continued). Data dictionary for coordinated monitoring programme for *Listeria monocytogenes* in certain ready-to-eat food categories at retail

Block 3: Appears if item "006" is " Smoked or gravad fish "						
Item Integer	**Variable**	**Constraint**	**Definition**	**Description and Particularity**	**Type**	**Values**
015	Subtype of the fish product	Mandatory	Subtype of smoked/gravad RTE fish that was sampled		List element	(Cold smoked fish); (Hot smoked fish); (Unknown smoked fish); (Gravad fish)
016	Fish species	Mandatory	Fish species	A list of commercial fish names (including the scientific names of the species) in the official languages of the MS required in Council Regulation (EC) No 104/2000 may also be consulted in choosing the species.	List element(s)	See separate list (Other – freetype here);
017	Preservatives and acidity regulators	Mandatory		Preservatives and acidity regulators as indicated on the label	List element(s)	See separate list (other – freetype here); (none added)
018	Date of testing for fish product on the arrival at the laboratory (starting time)	Mandatory	Date of laboratory testing	Date of primary testing in the laboratory. Detection and enumeration on the food sample should be started at the same time. Must not be earlier than date of sampling item [005].	Date	ISO 8601 (YYYY-MM-DD)
019	*Listeria monocytogenes* quantification on the arrival at the laboratory	Mandatory	Amount of *L. monocytogenes* detected in the sample (cfu/g)	Good example: "1.2 x 10 = 1 200" , "0" (0 means no colonies detected = less than 10 cfu/g).	Integer	Numeric
020	*Listeria monocytogenes* detection on the arrival at the laboratory	Mandatory	Presence in 25 g		Boolean	Yes No

Table continued overleaf.

Appendix A (continued). Data dictionary for coordinated monitoring programme for *Listeria monocytogenes* in certain ready-to-eat food categories at retail

Block 3: Appears if item "006" is " Smoked or gravad fish "						
Item Integer	**Variable**	**Constraint**	**Definition**	**Description and Particularity**	**Type**	**Values**
021	pH test result on the arrival at the laboratory	Mandatory		The result must be reported to the nearest 0.05 unit of pH. Value must be greater than or equal to 0.00 and less than or equal to 14.00	Integer	Numeric
022	Water activity (a_w) result on the arrival at the laboratory	Mandatory		The method shall be able of operating from 0.88 upwards. Value must be greater than or equal to 0.88 and less than or equal to 1.00.	Integer	Numeric
Block 4: Appears if item "006" is " Heat treated meat product "						
Item Integer	**Variable**	**Constraint**	**Definition**	**Description and Particularity**	**Type**	**Values**
023	Animal species of the origin of the meat product	Mandatory	Animal species of origin		List element	(Pork); (Beef); (Turkey); (Broiler); (Poultry); (Mixed); (Other – freetype here);
024	Type of the meat product	Mandatory	Type of the product	Cold, cooked meat product are meat products typically made with whole or large parts of anatomical or reformed structures such as cooked sliced ham and cooked chicken fillet	List element	(Sausage); (Pate); (Cold, cooked meat product)
024bis	Packaging place for meat	Mandatory	Packaging conditions of the meat product selected for sampling	Was the meat product packaged by the original producer or at retail?	List element	(Packaged by the producer);(Packaged at retail);(Unknown)

Table continued overleaf.

Appendix A (continued). Data dictionary for coordinated monitoring programme for *Listeria monocytogenes* in certain ready-to-eat food categories at retail

Block 5: Appears whatever the answer to item 006						
Item Integer	**Variable**	**Constraint**	**Definition**	**Description and Particularity**	**Type**	**Values**
025	Possible slicing	Mandatory	Is the product sliced		Boolean	Yes No
026	Packaging type	Mandatory	Type of packaging of the food product		List element	(Vacuum); (Modified atmosphere); (Normal atmosphere); (Other – freetype here)
027	Use by date	Mandatory	Final date for using the product as labelled	The use by date given by original producer or in case of re-packing at retail the final date for using the product. Date value must not be < 15 December 2009. Must not be earlier than date of sampling item [005].	Date	ISO 8601 (YYYY-MM-DD)
028	Production date	Optional	Production date if available		Date	ISO 8601 (YYYY-MM-DD)
029	Packaging date	Optional	Packaging date if available		Date	ISO 8601 (YYYY-MM-DD)
030	Country of production	Mandatory	Country of production	As ascertained with reference to the identification mark on packaging or commercial document	List element	ISO 3166-1-Alpha-2. All Member States + third countries
031	Storage temperature at retail	Mandatory	Temperature at retail (°C)	Value must be greater than or equal to 0 and less than or equal to 30.	Integer	Numeric

Table continued overleaf.

Appendix A (continued). Data dictionary for coordinated monitoring programme for *Listeria monocytogenes* in certain ready-to-eat food categories at retail

Block 5: Appears whatever the answer to item 006						
Item Integer	**Variable**	**Constraint**	**Definition**	**Description and Particularity**	**Type**	**Values**
032	Transport protocol	Mandatory	Transport in line with technical specifications	Can it be guaranteed that during the transport the sample was kept between 2 and 8 °C, if original storage temperature at retail was below 8 °C and remained free of external contamination and that the sample reached the laboratory in less than 48 hours?	Boolean	Yes No
033	Date of testing at the end of the shelf-life (starting time)	Mandatory	Date of laboratory testing	Date of primary testing in the laboratory. Detection and enumeration on the food sample should be started at the same time. Date should not be earlier than date at item 018.	Date	ISO 8601 (YYYY-MM-DD)
034	*Listeria monocytogenes* quantification result at the end of the shelf-life	Mandatory	Amount of *L. monocytogenes* detected in the sample (cfu/g)	Good example: "1.2 x 10 = 1 200", "0" (0 means no colonies detected = less than 10 cfu/g).	Integer	Numeric
035	*Listeria monocytogenes* detection at the end of the shelf-life	Mandatory	Presence in 25 g		Boolean	Yes No
036	Storage temperature at laboratory up to the end of shelf-life	Mandatory	Temperature during the laboratory storage (°C)	Values allowed: must be equal to or greater than 0 and less than or equal to 30.	Integer	Numeric (no decimals)
037	Suitability for human consumption at end of shelf-life	Optional	Suitable for human consumption on the basis of visual and smell evaluation		Boolean	Yes No

Table continued overleaf.

Appendix A (continued). Data dictionary for coordinated monitoring programme for *Listeria monocytogenes* in certain ready-to-eat food categories at retail

Definition of the data types used in this dictionary		
Name Text	**Definition**	**Example**
	Alphanumeric values	Ex. : 'Abcd1234'
Integer	rounded number values	Ex. : '1', '22', '333' , '44444'
Boolean	true or false value	e.g. YES or NO
Date	String corresponding to the following format: YYYY-MM-DD	Ex. : '2004-11-22'
List element	Must be only one of the value present in the 'Values' column	
List element(s)	Must be one or more values present in the 'Values' column	

Appendix B. Descriptive analysis concerning the factors appearing in the final models for both outcome variables

The following tables and figures provide further insights into the outcome variables in the sampled RTE food products. It is important to realize that the observed differences might be due to other factors, which is the reason for considering the multiple-factor analysis for the inferences in this report.

Table 9: Prevalence of *L. monocytogenes* in packaged hot or cold smoked or gravad fish samples, sampled for the *L. monocytogenes* baseline survey in the EU[(a)], 2010-2011

		No of samples	Samples contaminated with *L. monocytogenes* (%)	
			At time of sampling	At end of shelf-life
Subtype of the fish product	Unknown smoked fish	1 625	8.8	9.1
	Cold smoked fish	640	16.7	15.5
	Hot smoked fish	535	6.2	6.5
	Gravad fish	253	11.9	11.9
Number of antimicrobial preservatives and/or acidity regulators (AP/AR)	No reported AP/AR	2 915	9.7	9.8
	One AP/AR	83	4.8	4.8
	Two or more AP/AR	55	45.5	40
Possible slicing	Sliced	2 275	11.8	11.8
	Not sliced	778	5.7	5.7
Fish species	Salmon	1 859	12.3	12.4
	Mackerel	410	5.9	4.4
	Other fish	275	8.4	8
	Mixed fish	326	6.4	7.7
	Herring	183	9.3	8.7
Sampling season (modified variable)	Autumn	938	11.3	13.2
	Spring	675	7.4	7.7
	Summer	760	11.1	10.7
	Winter	680	10.7	8.1
EC 2073/2005 NSG	Included in EC 2073/2005 NSG	210	9.1	8.1
	Not included in EC 2073/2005 NSG	2 843	10.3	10.4

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.

Table 10: Summary statistics of 'Storage temperature at retail' by *L. monocytogenes* contamination (negative/positive/total) in packaged hot or cold smoked or gravad fish samples, at time of sampling and of 'Storage temperature at laboratory up to the end of shelf-life', by *L. monocytogenes* contamination at end of shelf-life, in fish samples, sampled for the *L. monocytogenes* baseline survey in the EU[(a)], 2010-2011

	Storage temperature at retail by contamination at time of sampling			Storage temperature at laboratory by contamination at end of shelf-life		
	Negative	**Positive**	**Total**	**Negative**	**Positive**	**Total**
Number	2 740	313	3 053	2 741	312	3 053
Mean	3.47	3.31	3.45	4.24	4.08	4.22
Standard deviation	1.83	1.43	1.79	1.27	1.36	1.28
Minimum	0	0	0	0	1	0
Lower whisker	0	0	0	4	2	4
First quartile	2	2	2	4	3	4
Median	3	3	3	4	4	4
Third quartile	4	4	4	4	4	4
Upper whisker	7	7	7	4	5	4
Maximum	25	10	25	8	8	8
Range (maximum–minimum)	25	10	25	8	7	8

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.

Table 11: Proportion (%) of packaged hot or cold smoked or gravad fish samples with a count of *L. monocytogenes* exceeding the level of 100 cfu/g, sampled for the *L. monocytogenes* baseline survey in the EU[(a)], 2010-2011

		No of samples	**Samples with *L. monocytogenes* count > 100 cfu/g (%)**	
			At time of sampling	**At end of shelf-life**
Type of retail outlet	Supermarket or small shop	3 004	0.9	1.6
	Other	44	0	4.6
	Speciality delis	3	33.3	33.3
	Street market/farmers' market	2	0	0
Type of retail outlet (modified variable)	Supermarket or small shop	3 004	0.9	1.6
	All other types of retail outlet	49	2	6.1
Possible slicing	Sliced	2 275	1.1	2
	Not sliced	778	0.4	0.8
Sampling season	Autumn	938	0.6	2.4
	Spring	675	0.7	0.4
	Summer	760	2	2.2
	Winter	680	0.4	1.5
EC 2073/2005 NSG	Included in EC 2073/2005 NSG	210	0.5	0
	Not included in EC 2073/2005 NSG	2 843	1	1.8

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.

Table 12: Prevalence of *L. monocytogenes* at the end of shelf-life, in packaged heat-treated meat product samples, sampled for the *L. monocytogenes* baseline survey in the EU[(a)], 2010-2011

		No of samples	Samples contaminated with *L. monocytogenes* (%)
Type of the meat product	Sausage	780	1.8
	Pâté	203	4.9
	Cold, cooked meat product	2 547	1.9
Packaging type	Normal atmosphere	548	2.9
	Modified atmosphere	2 001	1.7
	Vacuum	888	2.3
	Other	93	3.2
Packaging type (modified variable)	Modified atmosphere	2 001	1.7
	All other packaging types	1 529	2.6
Possible slicing	Sliced	3 005	2.1
	Not sliced	525	1.5

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.

Table 13: Proportion (%) of packaged heat-treated meat product samples with a count of *L. monocytogenes* exceeding the level of 100 cfu/g, sampled for the *L. monocytogenes* baseline survey in the EU[(a)], 2010-2011

		No of samples	Samples with *L. monocytogenes* count > 100 cfu/g (%)
Animal species of the origin of the meat product	Pork	2 566	0.3
	Poultry	210	1
	Broiler	92	2.2
	Turkey	232	0.4
	Beef	105	1
	Mixed	308	0.3
	Goose	1	0
	Other	16	0
Animal species of the origin of the meat product (modified variable)	Avian species	535	0.9
	All other species	2 995	0.3
Possible slicing	Sliced	3 005	0.5
	Not sliced	525	0.2

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.

Table 14: Summary statistics of 'Remaining shelf-life', for packaged heat-treated meat product samples with *L. monocytogenes* counts exceeding and not exceeding the level of 100 cfu/g at end of shelf-life, sampled for the *L. monocytogenes* baseline survey in the EU[(a)], 2010-2011

	Samples with *L. monocytogenes* count		**All samples**
	≤ 100 cfu/g	**> 100 cfu/g**	
Number	3 515	15	3 530
Mean	19.46	29.53	19.51
Standard deviation	20.01	20.87	20.02
Minimum	0	5	0
Lower whisker	0	5	0
First quartile	10	18.5	10
Median	15	24	15
Third quartile	23	31	23
Upper whisker	42	46	42
Maximum	427	86	427
Range (maximum–minimum)	427	81	427

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.

Appendix C. Some details on statistical methodology and issues and on the interpretation of model findings

Model building

In order to take the hierarchical structure into account, GEE models were fitted. The complexity of the model increases as the number of explanatory variables increases, especially in the case of nominal categorical variables with many categories. This condition of many categorical variables may also cause sparseness and may lead to computational/convergence problems.

As there were many explanatory variables in the dataset, a semi-automatic procedure of variable selection and reduction was considered. One effective way to do the variable reduction in case of a binary outcome is by using automatic selection procedures for logistic regression. A selected sub-model resulting from an automated logistic regression procedure needs to be examined further in order to get a fine-tuned final model. Indeed, as logistic regression typically leads to consistent estimates but too small estimated standard errors in case of hierarchically clustered data, the selected logistic regression model, refit as a GEE model, can be further reduced by deleting those factors which are no longer significant in the corresponding GEE model (see, for example, Aerts et al., 2002).

Therefore, the following procedure was applied:

1. automated model selection–all subsets selection approach–using ordinary logistic regression (possibly indicating too many significant effects as clustering/correlation has been ignored);

2. refitting the selected model by GEE and reducing that model to get a final model;

3. further sensitivity analysis, as previously described.

For more details, the reader is referred to the External Report.

Some notes on the interpretation of model findings: associations expressed as odds ratios

The regression coefficients (βs) are related to the ORs as follows: the exponentiated value of a regression coefficient $\exp(\beta)$ equals to the OR associated with a one-unit increase in the exposure factor. This applies to factors that do not participate in any interaction terms.

A categorical variable with more than two categories is represented by a set of indicator variables (*c-1* indicator variables in case of *c* categories). An indicator variable takes the value of one or zero with respect to the presence or absence of a certain category of a categorical factor. For example, the variable 'Fish species' had five categories: 'Salmon', 'Herring', 'Mackerel', 'Mixed fish' and 'Other fish'. One reference or baseline category needed to be chosen, e.g. 'Salmon'. The outcome in this reference category was then compared with the outcome in the other categories. However, the choice of the reference category did not influence the model building and model inference. Four indicator variables were created:

Table 15: Indicator variables for 'Fish species'

Fish species	Indicator_I_1: Herring	Indicator_I_2: Mackerel	Indicator_I_3: Mixed fish	Indicator_I_4: Other fish
Salmon	0	0	0	0
Herring	1	0	0	0
Mackerel	0	1	0	0
Mixed fish	0	0	1	0
Other fish	0	0	0	1

Consider the following logistic regression model for the prevalence $\pi = \Pr(P = 1)$ of *L. monocytogenes* ($P = 1$ for a positive outcome, and 0 otherwise) and with 'Fish species' as an investigated factor

$$\ln\left\{\frac{\pi}{1-\pi}\right\} = \beta_0 + \beta_1 I_1 + \beta_2 I_2 + \beta_3 I_3 + \beta_4 I_4$$

For the 'Salmon' fish species (the reference category), the model simplifies to the intercept only (as all indicator variables are equal to 0)

$$\ln\left\{\frac{\pi(\text{Salmon})}{1-\pi(\text{Salmon})}\right\} = \beta_0$$

whereas for 'Mackerel' the model reduces to (as only the second indicator variable equals 1)

$$\ln\left\{\frac{\pi(\text{Mackerel})}{1-\pi(\text{Mackerel})}\right\} = \beta_0 + \beta_2$$

Consequently, the parameter β_2 expresses the change in the log odds $\ln\left\{\frac{\pi}{1-\pi}\right\}$ and hence in the prevalence when looking at 'Mackerel' as compared with the baseline or reference category (in this case, 'Salmon'). After some basic manipulations, $\exp(\beta_2)$ is the OR for a positive outcome, when comparing 'Mackerel' with 'Salmon' (as a two by two table with positive/negative classification in one direction and 'Mackerel'/'Salmon' in the other direction):

$$\exp(\beta_2) = \frac{\pi(\text{Mackerel}) \times (1-\pi(\text{Salmon}))}{(1-\pi(\text{Mackerel})) \times \pi(\text{Salmon})}$$

This can be done in the same way for the 'Herring', 'Mixed fish' and 'Other fish' species, always in comparison with the baseline 'Salmon'.

When interaction is present, the OR between a particular factor and the outcome varies according to and depends upon the value of the other factor involved in the interaction term. If the interaction between factors is significant, the main effects are no longer summarizing the effect of the factors. Indeed, the effect of one factor varies with the value of the other factor, and one needs to look at the main effects together with the interaction effect. It is common practice to keep the main effects in the model even if they are not significant.

The interaction between two variables can be positive (their joint role increases the effect) or negative (their joint role decreases the effect).

Appendix D. Statistical models for the factors related to the modelled outcomes and sensitivity analysis

Analysis of factors related to the prevalence of *L. monocytogenes*-contaminated fish samples at time of sampling

Table 16: Detailed outcomes of final GEE model (with associated ORs) for prevalence of *L. monocytogenes* in packaged hot or cold smoked or gravad fish samples at time of sampling, in the EU[(a)], 2010-2011

Model term		OR	95 % CI		*P*-value
Intercept		0.13	0.08	0.22	< 0.0001
Subtype of the fish product[(b)]	Gravad fish	0.72	0.45	1.16	0.18
	Hot smoked fish	0.54	0.33	0.89	0.02
	Unknown smoked fish	0.57	0.42	0.75	0.001
Fish species[(c)]	Herring	1.09	0.56	2.12	0.81
	Mackerel	0.52	0.29	0.93	0.03
	Mixed Fish	0.46	0.27	0.79	0.005
	Other Fish	0.80	0.48	1.35	0.41
Number of antimicrobial preservatives and/or acidity regulators (AP/AR)[(d)]	One AP/AR	0.55	0.20	1.49	0.24
	Two or more AP/AR	7.89	4.33	14.39	< 0.0001
Possible slicing[(e)]		1.59	1.02	2.48	0.04
EC 2073/2005 NSG[(f)]		0.53	0.21	1.33	0.17
EC 2073/2005 NSG*Fish species	Herring	0.78	0.12	5.08	0.79
	Mackerel	6.76	1.86	24.61	0.004
	Mixed Fish	1.41	0.27	7.26	0.68
	Other Fish	0.70	0.12	4.15	0.69

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this analysis.
(b): The baseline category for 'Subtype of the fish product' is 'Cold smoked fish'.
(c): The baseline category for 'Fish species' is 'Salmon'.
(d): The baseline category for 'Number of antimicrobial preservatives and/or acidity regulators (AP/AR)' is 'No reported AP/AR'.
(e): The baseline category for 'Possible slicing' is 'Not sliced'.
(f): The baseline category for 'EC 2073/2005 NSG' is 'Not included in EC 2073/2005 NSG'.

Sensitivity analysis

a. The sample numbers had been submitted in numbers designed around approximate weightings per individual MS, but further weighting was also considered (for details the reader is referred to the External Report). Concerning the comparison between the OR estimates from the weighted analyses and the unweighted analyses: most of the factors in the final model were quite insensitive to the weighing. But there were some remarkable differences:

   - the OR corresponding to the variable 'Possible slicing' was no longer significant in the weighted analyses.
   - the OR for 'Gravad fish' as compared with 'Cold smoked fish' was significantly lower than 1, when using weights related to population size.

   As both weights are merely proxy weights for the unknown true weight (that would correct for over- or under-representation), interpretation of these differences is not straightforward. The major

conclusion is that one should be careful with formulating strong statements about those factors that are unstable across such unweighted and weighted analyses.

b. Logistic regression with Firth's correction method for sparseness: the results of the GEE model are very close to those obtained by the model, using the Firth method. This indicates and confirms that there are no major sparseness issues in the final GEE model.

c. GEE analysis for final model with continuous no-growth probability (model results not shown) shows that most factors and interactions behave quite robustly with respect to the use of either the binary 'EC 2073/2005 NSG' variable or the continuous variable estimating the probability of no-growth of *L. monocytogenes*, whereas one factor seems to be more sensitive:

- Stable: 'Subtype of the fish product', 'Number of antimicrobial preservatives and/or acidity regulators (AP/AR)', 'Possible slicing';
- Sensitive: 'Fish species' (no longer significant, main effect nor interaction effect).

Goodness-of-fit test

Goodness-of-fit test was performed using the Hosmer and Lemeshow Chi-Square test. The result showed a lack of fit (*P*-value = 0.02). As explained in the External Report, caution is necessary in the interpretation of the results of this test, which has been developed for logistic regression only and not for GEE or Firth's method. Consequently, it is not known what effect the clustered nature or the sparse nature of the data have on the validity of the test.

Multicollinearity analysis

The VIF values for the potentially intercorrelated factors from the final model are presented in Table 17. This analysis showed that multicollinearity was not important for the full model since all the VIF values were small (considerably less than 10).

Table 17: Variance Inflation Factor (VIF) values for the variables in the final model for prevalence of *L. monocytogenes* in packaged hot or cold smoked or gravad fish samples, at time of sampling, in the EU[(a)], 2010-2011

Variable	VIF
Subtype of the fish product	1.82
Fish species	3.47
Number of antimicrobial preservatives and/or acidity regulators (AP/AR)	5.97
Possible slicing	1.50
EC 2073/2005 NSG	2.55

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this analysis.

Analysis of factors related to the prevalence of *L. monocytogenes*-contaminated fish samples at end of shelf-life

Table 18: Detailed outcomes of final GEE model (with associated ORs) for prevalence of *L. monocytogenes* in packaged hot or cold smoked or gravad fish samples at end of shelf-life, in the EU[(a)], 2010-2011

Model term		OR	95 % CI		*P*-value
Intercept		0.15	0.07	0.32	< 0.0001
Sampling season[(b)]	Autumn	1.76	1.23	2.52	0.002
	Spring	0.98	0.65	1.47	0.91
	Summer	1.39	0.95	2.03	0.09
Subtype of the fish product[(c)]	Gravad fish	0.86	0.53	1.40	0.55
	Hot smoked fish	0.61	0.38	0.98	0.04
	Unknown smoked fish	0.62	0.45	0.86	0.004
Fish species[(d)]	Herring	1.05	0.55	2.01	0.88
	Mackerel	0.33	0.17	0.64	0.001
	Mixed fish	0.65	0.40	1.06	0.08
	Other fish	0.80	0.48	1.34	0.40
Number of antimicrobial preservatives and/or acidity regulators (AP/AR)[(e)]	One AP/AR	0.60	0.20	1.77	0.36
	Two or more AP/AR	7.15	3.61	14.17	< 0.0001
Possible slicing[(f)]		1.39	0.91	2.12	0.13
Storage temperature at laboratory up to the end of shelf-life[(g)]		0.91	0.82	1.02	0.10
EC 2073/2005 NSG[(h)]		0.06	0.01	0.57	0.01
EC 2073/2005 NSG*fish species	Herring	0.26	0.02	2.81	0.27
	Mackerel	7.26	1.68	31.35	0.01
	Mixed fish	0.47	0.05	4.59	0.52
	Other fish	0.15	0.01	2.08	0.16
Storage temperature at laboratory up to the end of shelf-life * EC 2073/2005 NSG		1.84	1.12	3.02	0.02

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this analysis.
(b): The baseline category for 'Sampling season' is 'Winter'.
(c): The baseline category for 'Subtype of the fish product' is 'Cold smoked fish'.
(d): The baseline category for 'Fish species' is 'Salmon'.
(e): The baseline category for 'Number of antimicrobial preservatives and/or acidity regulators (AP/AR)' is 'No reported AP/AR'.
(f): The baseline category for 'Possible slicing' is 'Not sliced'.
(g): For samples with 'Storage at 1 °C higher' compared with samples stored at a given temperature.
(h): The baseline category for 'EC 2073/2005 NSG' is 'Not included in EC 2073/2005 NSG'.

Sensitivity analysis

a. Concerning the comparison between the OR estimates from the weighted analyses and the unweighted analyses: analyses of results across different weighting schemes were consistent and the model was quite robust, regardless of the weighting scheme.

b. Logistic regression with Firth's correction method for sparseness: confirms the stability of the results across the two different models.

c. GEE analysis for final model with continuous no-growth probability (model results not shown): most factors were quite robust for this modification, except for 'Fish species', for which 'Herring' but not 'Mackerel' had an OR significantly different from 1 (OR = 3.09,

P-value = 0.04) and a significant interaction (*P*-value = 0.01) with the continuous no-growth probability variable. Also, the variable 'Storage temperature at laboratory up to the end of shelf-life' and its interaction with the variable indicating the probability of no-growth was not significant.

Goodness-of-fit test

Goodness-of-fit test was performed using the Hosmer and Lemeshow Chi-Square test. The result (*P*-value = 0.19) shows that there was no lack of fit in the model since the *P*-value was larger than 5 %. The null hypothesis that the final model is an appropriate model could not be rejected, or, in other words there was no evidence of any lack of fit.

Multicollinearity analysis

All VIF values were small (considerably less than 10). So there did not seem to be any problems with multicollinearity.

Table 19: Variance Inflation Factor (VIF) values for the variables in the final model for prevalence of *L. monocytogenes* in packaged hot or cold smoked or gravad fish samples at end of shelf-life, in the EU[(a)], 2010-2011

Variable	VIF
Sampling season	1.04
Subtype of the fish product	1.88
Fish species	3.55
Number of antimicrobial preservatives and/or acidity regulators (AP/AR)	6.36
Possible slicing	1.75
EC 2073/2005 NSG	2.63
Storage temperature at laboratory up to the end of shelf-life	1.08

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this analysis.

Analysis of factors related to the proportion of fish samples with an *L. monocytogenes* count > 100 cfu/g at time of sampling

Table 20: Detailed outcomes of final GEE model (with associated ORs) for proportion of packaged hot or cold smoked or gravad fish samples with an *L. monocytogenes* count exceeding the level of 100 cfu/g at time of sampling, in the EU[(a)], 2010-2011

Model term		OR	95 % CI		*P*-value
Intercept		0.00	0.00	0.01	< 0.0001
Sampling season[(b)]	Autumn	1.35	0.34	5.44	0.67
	Spring	1.65	0.39	6.92	0.49
	Summer	4.29	1.22	15.03	0.02
Possible slicing[(c)]		2.79	0.90	8.58	0.07
EC 2073/2005 NSG[(d)]		0.55	0.08	3.94	0.55

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this analysis.
(b): The reference category for 'Sampling season' is 'Winter'.
(c): The reference category for 'Possible slicing' is 'Not sliced'.
(d): The reference category for 'EC 2073/2005 NSG' is 'Not included in EC 2073/2005 NSG'.

Sensitivity analysis

a. Concerning the comparison between the OR estimates from the weighted analyses and the unweighted analyses: the factors in the final model were all insensitive to the weighting. So, all conclusions remained the same, regardless of the weighting scheme.

b. Logistic regression with Firth's correction method for sparseness: all estimates were in line with the GEE analysis results.

c. GEE analysis for final model with continuous no-growth probability: the final model was also quite insensitive compared to the results obtained using the binary 'EC 2073/2005 NSG' variable.

Goodness-of-fit test

A goodness-of-fit test was performed using the Hosmer and Lemeshow Chi-Square test. The result (*P*-value = 0.91) showed that there was no evidence for lack of fit in the model since the *P*-value was larger than 5 %.

Multicollinearity analysis

The VIF values were small (considerably less than 10).

Table 21: Variance Inflation Factor (VIF) values for the variables in the final model for proportion of packaged hot or cold smoked or gravad fish samples with an *L. monocytogenes* count exceeding the level of 100 cfu/g at time of sampling, in the EU[(a)], 2010-2011

Variable	VIF
Sampling season	1.03
Possible slicing	1.27
EC 2073/2005 NSG	2.36

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this analysis.

Analysis of factors related to the proportion of fish samples with an *L. monocytogenes* count > 100 cfu/g at end of shelf-life

Table 22: Detailed outcomes of final GEE model (with associated ORs) for proportion of packaged hot or cold smoked or gravad fish samples with an *L. monocytogenes* count exceeding the level of 100 cfu/g at end of shelf-life, in the EU[(a)], 2010-2011

Model term		OR	95 % CI		*P*-value
Intercept		0.01	0.00	0.02	< 0.0001
Type of retail outlet[(b)]		4.29	1.29	14.22	0.02
Sampling season[(c)]	Autumn	1.39	0.64	3.00	0.41
	Spring	0.28	0.08	1.02	0.05
	Summer	1.45	0.66	3.21	0.36
Possible slicing[(d)]		2.55	1.07	6.05	0.03

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this analysis.
(b): Refers to the modified variable (two levels). The baseline category for 'Type of retail outlet' is 'Supermarket or small shop'.
(c): The baseline category for 'Sampling season' is 'Winter'.
(d): The baseline category for 'Possible slicing' is 'Not sliced'.

Sensitivity analysis

a. Weighted analyses versus unweighted analyses: the significance of the ORs for the variables 'Sampling season' and 'Possible slicing' changed for weighted analysis compared with unweighted analysis. The corresponding ORs were still quite similar. As both sets of weights are merely proxy weights for the unknown true weights (that would correct for over- or under-representation), interpretation of these differences is not straightforward. The major conclusion is that one should be careful with formulating strong statements about those factors that are unstable across such unweighted and weighted analyses.

b. Logistic regression with Firth's correction method for sparseness: the results of the GEE model were very close to those obtained with the Firth method. This indicated and confirmed that there were no major sparseness issues in the final GEE model.

c. Exact logistic regression for final model: exact logistic regression was performed using the variables selected for the final model with the binary 'EC 2073/2005 NSG' variable. Most of the ORs were similar to those of the GEE analysis. The main difference was that the ORs corresponding to the variables 'Type of retail outlet' and 'Sampling season': 'Spring' were no longer significant, but the exact logistic regression ignores the hierarchical structure in the data.

d. GEE analysis for final model with continuous no-growth probability: Although the final model did not contain the binary 'EC 2073/2005 NSG' variable, an analysis was done using the continuous no-growth probability. The results showed that the factors were not influenced by the inclusion of the continuous no-growth variable, which was not significantly associated with the outcome.

Goodness-of-fit test

Goodness-of-fit test was performed using the Hosmer-Lemeshow Chi-Square test. The result showed that there was no lack of fit in the final model since the *P*-value was larger than 5 %.

Multicollinearity analysis

The VIF values calculated for the multicollinearity analysis among potentially associated factors that related to the above final model are presented in Table 23. This analysis showed that multicollinearity was not important for the full model since all the VIF values were small (considerably less than 10).

Table 23: Variance Inflation Factor (VIF) values for the variables in the final model for proportion of packaged hot or cold smoked or gravad fish samples with an *L. monocytogenes* count exceeding the level of 100 cfu/g at end of shelf-life, in the EU[(a)], 2010-2011

Variable	VIF
Type of retail outlet	3.05
Sampling season	1.04
Possible slicing	1.25

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this analysis.

Analysis of factors related to the prevalence of *L. monocytogenes*-contaminated meat product samples at end of shelf-life

Table 24: Detailed outcomes of final GEE model (with associated ORs) for prevalence of *L. monocytogenes* in packaged heat-treated meat product samples, at end of shelf-life, in the EU[(a)], 2010-2011

Model term		OR	95 % CI		*P*-value
Intercept		0.01	0.01	0.03	< 0.0001
Type of the meat product[(b)]	Pâté	2.91	1.39	6.10	0.005
	Sausage	0.97	0.52	1.82	0.93
Possible slicing[(c)]		2.13	0.94	4.83	0.07
Packaging type[(d)]		0.60	0.36	0.99	0.048

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this analysis.
(b): The baseline category for 'Type of the meat product' is 'Cold, cooked meat product'.
(c): The baseline category for 'Possible slicing' is 'Not sliced'.
(d): Refers to the modified variable (two levels). The baseline category for 'Packaging type' is 'All other packaging types'.

Sensitivity analysis

a. Concerning the comparison between the OR estimates from the weighted analyses and the unweighted analyses: the association of *L. monocytogenes* prevalence with 'Type of the meat product' was consistent in all analyses. However, for the other two factors, results were significant or non-significant, depending on the use of weights or not. The OR associated with 'Possible slicing' was borderline non-significant (*P*-value = 0.07) for the unweighted analysis while it was significant (*P*-value = 0.03 and *P*-value = 0.01, depending on the weighting scheme) for the weighted analysis. Conversely, the OR corresponding to 'Packaging type' was significant (*P*-value = 0.048) in the unweighted analysis, while it was not significant in the analyses using weights (*P*-value = 0.10 or 0.09, depending on the weighting scheme).

   As both weighting schemes are merely proxies for the unknown true weights (that would correct for over- or under-representation), interpretation of these differences is not straightforward. The major conclusion is that one should be careful with formulating strong statements about those factors that are unstable across such unweighted and weighted analyses.

b. Logistic regression with Firth's correction method for sparseness: the *P*-values were almost the same as in the GEE model and the ORs were of the same magnitude as the ORs of the GEE model. This indicates and confirms that there were no major sparseness issues in the final GEE model.

Goodness-of-fit test

The goodness-of-fit test (Hosmer-Lemeshow) showed that there was no lack of fit in the model since the *P*-value was higher than the 5 % significance level (*P*-value = 0.48).

Multicollinearity analysis

The VIF values between the factors in the final model above are given in Table 25. All values are very small (considerably less than 10), so we conclude that no problems related to multicollinearity occurred.

Table 25: Variance Inflation Factor (VIF) values for the variables in the final model for prevalence of *L. monocytogenes* in packaged heat-treated meat product samples at end of shelf-life, in the EU[(a)], 2010-2011

Variable	VIF
Type of the meat product	2.25
Possible slicing	1.80
Packaging type	1.13
Storage temperature at laboratory up to the end of shelf-life	1.08

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this analysis.

Analysis of factors related to the proportion of meat product samples with a count of *L. monocytogenes* > 100 cfu/g at end of shelf-life

Table 26: Detailed outcomes of final GEE model (with associated ORs) for proportion of packaged heat-treated meat product samples, with an *L. monocytogenes* count exceeding 100 cfu/g at end of shelf-life, in the EU[(a)], 2010-2011

Model term	OR	95 % CI		*P*-Value
Intercept	0.00	0.00	0.02	< 0.0001
Animal species of the origin of the meat product[(b)]	0.35	0.13	0.97	0.04
Possible slicing[(c)]	2.61	0.33	20.53	0.36
Remaining shelf-life[(d)]	1.010	1.005	1.016	0.0002

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this analysis.
(b): Refers to the modified variable (two levels). The baseline category for 'Animal species of the origin of the meat product' is 'Avian species'.
(c): The baseline category for 'Possible slicing' is 'Not sliced'.
(d): For samples with 'One additional day of remaining shelf-life' compared with samples with a given 'Number of days of remaining shelf-life'

Sensitivity analysis

a. Concerning the comparison between the OR estimates from the weighted analyses and the unweighted analyses, most of the ORs were quite insensitive to the weighting. The OR associated with the 'Animal species of the origin of the meat product' was non-significant (*P*-value = 0.06) in one of the weighted analyses.

b. Logistic regression with Firth's correction method for sparseness: the results obtained with the model fit with the method of Firth and the GEE model were quite similar. In this case, the OR associated with the 'Animal species of the origin of the meat product' was significant in both weighted and unweighted analyses. As no major differences in the results obtained by the two models could be readily observed, it was concluded that there were no major sparseness problems in the variables of the final model.

Goodness-of-fit test

The goodness-of-fit test (Hosmer-Lemeshow) showed that there was lack of fit in the model since the *P*-value was lower than 5 % (*P*-value = 0.04). As explained in the External Report, caution is necessary in the interpretation of the results of this test, which has been developed for logistic

regression only and not for GEE or Firth's method. Consequently, it is not known what effect the clustered or the sparse nature of the data have on the validity of the test.

Multicollinearity analysis

The VIF between the factors in the final model above are given in Table 27. All values were very small (considerably less than 10), so it was concluded that no problems related to multicollinearity occurred.

Table 27: Variance Inflation Factor (VIF) values for the variables in the final model for proportion of packaged heat-treated meat product samples with an *L. monocytogenes* count exceeding the level of 100 cfu/g at end of shelf-life, in the EU[(a)], 2010-2011

Variable	VIF
Animal species of the origin of the meat product	1.63
Possible slicing	1.28
Remaining shelf-life	1.05

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this analysis.

ABBREVIATIONS

a_w	Water activity
AP/AR	Antimicrobial preservatives and/or acidity regulators
CI	Confidence Interval
cfu	Colony forming units
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EU	European Union
FPE	Food Processing Environment
GEE	Generalized Estimating Equations
HACCP	Hazard Analysis and Critical Control Points
ML	Maximum Likelihood
MS	Member State
NSG	Not supporting the growth
OR	Odds Ratio
pH	p[H], often written as, pH, is a measure of hydrogen ion concentration; a measure of the acidity or alkalinity of a solution.
RTE	Ready-to-eat
VIF	Variance Inflation Factor