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 A: *Listeria monocytogenes* prevalence estimates[1]

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ABSTRACT

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 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 and one country not belonging to the European Union. The fish batch samples were analysed on 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 *Listeria monocytogenes*, in addition to the determination of the *Listeria monocytogenes* counts. The prevalence across the entire European Union in fish samples at the time of sampling was 10.4 % and at the end of shelf-life was 10.3 %, while for meat and cheese samples at the end of shelf-life these prevalences were 2.07 % and 0.47 %, respectively. The European Union level proportion of samples with a *Listeria monocytogenes* count exceeding the level of 100 cfu/g at the end of shelf-life was 1.7 %, 0.43 % and 0.06 % for fish, meat and cheese samples, respectively, while for fish at the time of sampling it was 1 %. Summaries of the counts of *Listeria monocytogenes* in the examined samples are also presented.

KEY WORDS

Listeria monocytogenes, ready-to-eat food, fish, meat, cheese, prevalence, European Union.

[1] On request from the European Commission, Question No EFSA-Q-2011-00137, approved on 27 May 2013.

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[3] Acknowledgement: EFSA wishes to thank the members of the Working Group on the Analysis of the baseline survey on the prevalence of *Listeria monocytogenes* in certain RTE foods: Apostolos Angelidis, Lieven De Zutter, Nathalie Gnanou-Besse, Mats Lindblad, Micheál O'Mahony, Mieke Uyttendaele and Martin Wagner for the preparatory work on this scientific output and the hearing expert: Marc Aerts, the reviewer Konstantinos Koutsoumanis and EFSA staff: Frank Boelaert, Pia Mäkelä, Marios Georgiadis and Winy Messens for the support provided to this scientific output.

Suggested citation: 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

Available online: www.efsa.europa.eu/efsajournal

SUMMARY

In the European Union (EU), listeriosis is a relatively rare but serious food-borne illness in humans, with high morbidity, hospitalisation and mortality in vulnerable populations. The bacterial genus *Listeria* currently comprises 10 species, but human cases of listeriosis are almost exclusively caused by the species *Listeria monocytogenes* (*L. monocytogenes*). *Listeria* species are ubiquitous organisms that are widely distributed in the environment, especially in plant matter and soil. The principal reservoirs of *Listeria* are soil, forage and surface water. The main route of transmission to humans is believed to be through consumption of contaminated food. The bacterium can be found in raw foods and in processed foods that are contaminated during and/or after processing. The fact that *L. monocytogenes* is able to multiply in various foods at temperatures as low as 2 to 4 °C makes the occurrence of *L. monocytogenes* in ready-to-eat (RTE) foods with a relatively long shelf-life, such as fishery products, heat-treated meat products and RTE cheese, of particular concern.

In order to estimate at the EU level the prevalence and level of *L. monocytogenes* in packaged hot or cold smoked or gravad fish, packaged heat-treated meat products and soft and semi-soft cheeses (excluding fresh cheeses), an EU wide *L. monocytogenes* baseline survey was conducted at retail. The foods to be sampled were randomly selected from the customer display in the outlet and each sample weighed at least 100 g. The survey was designed to yield estimates at the EU level only and not at the Member State level.

Sampling took place between January 2010 and January 2012. A total of 3 053 batches of packaged 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 EU Member States, plus Norway. For fish, two samples were collected from each sampled batch and one was analysed on arrival at the laboratory (at the time of sampling) and the other one was analysed at the end of shelf-life. For the meat products and cheese samples one sample was taken from the selected batch and was 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.

The EU prevalence of *L. monocytogenes*-contaminated fish samples at time of sampling was 10.4 % while at the end of shelf-life it was 10.3 %. The EU level proportion of samples exceeding the food safety limit of 100 colony forming units (cfu)/g at sampling was 1.0 % while for fish at the end of shelf-life it was 1.7 %. Among meat products, the EU prevalence of *L. monocytogenes*-contaminated samples at the end of shelf-life was 2.07 % while the EU level proportion of samples exceeding the level of 100 cfu/g was 0.43 %. The EU prevalence of *L. monocytogenes*-contaminated cheese samples at the end of shelf-life was 0.47 % while the EU level proportion of samples exceeding the level of 100 cfu/g was 0.06 %.

Considering the enumeration test alone, the proportion of fish samples considered positive, defined as a *L. monocytogenes* count of 10 cfu/g or more, was 2.2 % and 3.2 % at the time of sampling and at the end of shelf-life, respectively. Of the 66 fish samples at time of sampling having a count of 10 cfu/g or more, 29 samples contained *L. monocytogenes* exceeding the level of 100 cfu/g. At the end of shelf-life of the 99 fish samples with a count of 10 cfu/g or more, 52 samples contained *L. monocytogenes* exceeding the level of 100 cfu/g. The proportion of packaged heat-treated meat products samples considered negative by the enumeration test was 99.1 % at the end of shelf-life whereas 0.9 % had a positive enumeration result. Of the 32 meat products samples at the end of shelf-life having a count of 10 cfu/g or more, 15 samples contained *L. monocytogenes* exceeding the level of 100 cfu/g. Enumeration showed that only four soft or semi-soft cheese products were positive, and in only two of these products did the *L. monocytogenes* count exceed 100 cfu/g at the end of shelf-life.

RTE foods with a relatively long shelf-life, such as fishery and heat-treated meat products, and ready-to-eat cheese are considered an important food-borne source of human *L. monocytogenes* infections in the EU. The risk for human health arises from exposure to *L. monocytogenes* in such foods and in particular foods containing *L. monocytogenes* exceeding the level of 100 cfu/g. In this survey a low

proportion of fish samples contained *L. monocytogenes* at levels exceeding the food safety limit of 100 cfu/g at the end of shelf-life. This is of concern to public health as the risk of human listeriosis increases with increasing numbers of ingested cells. The proportion of cooked meat samples exceeding the level of 100 cfu/g was very low and soft and semi-soft cheeses samples exceeding this level were rare. However, even a very low to rare proportion of samples exceeding the level of 100 cfu/g may raise concern for public health.

Good manufacturing practices, appropriate cleaning, sanitation and hygiene programs and effective temperature control throughout the food production, distribution and storage chain are required for prevention of contamination or inhibition of growth of *L. monocytogenes* to levels exceeding 100 cfu/g in foods that may pose a *L. monocytogenes* risk. The surveyed foods were RTE and therefore intended to be consumed without any further heat treatment. The findings indicate the ongoing presence of *L. monocytogenes* in such foods. All food business operators and consumers should keep the temperatures of their refrigerators low, in order to limit potential growth of *L. monocytogenes* if this is present in RTE products.

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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 the EFSA proposal, 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 A) describes the results of a baseline survey carried out in the EU to estimate the prevalence of *L. monocytogenes* in certain ready-to-eat (RTE) foods at 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-not at Member State level. 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. Thus, this survey approaches the concept of risk arising from this hazard to which the EU consumer of these products might be exposed. However this survey targets *L. monocytogenes* in RTE food products previously shown to be at risk of contamination, and does 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 Member States (MSs) 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 MSs (EFSA, 2009b). Illness was often severe and mortality was reported at 20 %.

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 submitted to EFSA (later referred to as the External Report) on the Statistical analysis of the *L. monocytogenes* EU wide baseline survey in certain RTE foods. Part A: *L. monocytogenes* prevalence (Rakhmawati et al., 2013) and prepared by an EFSA contractor reports on EU level prevalence analyses and on MS specific data and descriptive statistics. Therefore, this report should be read in parallel with that External Report.

The 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 MSs 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 subsequent Part B report on the analyses of the baseline survey on the prevalence of *L. monocytogenes* in certain RTE foods will present the analyses of risk factors related to the prevalence of contaminated foods, the predictive models for the microbial growth of *L. monocytogenes* under various storage conditions, and the predictive models for compliance with *L. monocytogenes* food safety criteria in foods. The Part B report will be published at a later date.

[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.

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 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.

Twenty-six EU MSs, 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.

2. Definitions

Basic definitions to be considered in the scope of this baseline survey were provided in Commission Decision 2010/678/EU. For the purpose of this document, the following definitions apply:

At sampling: The survey specifications required the batches of the fish food category to be analysed twice, with one analysis performed immediately after sampling. There was, however an allowance for that analysis to begin within 24 hours after sampling and, within the context of normal working around weekends, further time-lags from sampling might have occurred. Nevertheless, the results obtained represent analysis at a point in the shelf-life of the product before the end of shelf-life, which was around the time of sampling. The results arising from this analysis are referred to in this report as results 'at sampling'.

At the end of shelf-life: The survey specifications required all three food categories to be analysed at the end of shelf-life, following storage in the laboratory between sampling and the end of shelf-life. Allowances were again provided to take into account whether the end of shelf-life coincided with a weekend or public holiday, whereby analysis could begin on the last working day prior to the end of shelf-life. The results arising from this analysis are referred to in this report as results 'at the end of the shelf-life'.

Batch: A group or set of identifiable products obtained from a given process under practically identical circumstances and produced in a given place within one defined production period (Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs).[6]

Cheeses

- **Soft cheeses** – cheeses that have a percentage moisture, on a fat-free basis, higher than 67 % (CAC, 2008).
- **Semi-soft cheeses** – cheeses that have a texture which is only slighter harder than the soft cheese category. These cheeses have a percentage moisture, on a fat-free basis, ranging from 62 to 67 %. Semi-soft cheeses are characterized by their firm but elastic feel.
- **Ripened cheeses** – cheeses which are not ready for consumption shortly after manufacture but which must be held for such time, at such temperature, and under such other conditions as will result in the necessary biochemical and physical changes characterizing the cheese in question (CAC, 2008).
- **Mould-ripened cheeses** – ripened cheeses in which the ripening has been accomplished primarily by the development of characteristic mould growth throughout the interior and/or on the surface of the cheese (CAC, 2008).

[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.

- **Smear-ripened cheeses** – ripened cheeses in which during or after ripening, the cheese rind is treated or naturally colonized with desired cultures of microorganisms, for instance *Penicillium candidum* or *Brevibacterium linens.* The resulting layer or **smear** forms a part of the rind (CAC, 2008).
- **Brine-matured cheeses** – cheeses matured and stored in brine until they are sold or packed.
- **Fresh cheeses** – curd-style cheeses which do not undergo any ripening, for example cottage cheese, mozarella, ricotta, and quark. *Fresh cheeses are not included in this survey.*

Compliance with microbiological criteria: Obtaining satisfactory or acceptable results set in Annex I of the Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs, when testing against the values set for the criteria through the taking of samples, the conduct of analyses and the implementation of corrective action, in accordance with food law and the instructions given by the competent authority.

Contamination: Means the presence or introduction of a hazard (Regulation (EC) No 852/2004 of the European Parliament and of the Council on the hygiene of foodstuffs).[7]

Country of production: The country indicated in the identification mark referred to in Article 1 of Regulation (EC) No 853/2004 of the European Parliament and of the Council laying down specific hygiene rules for food of animal origin.[8]

Food safety criterion: Criterion defining the acceptability of a product or a batch of foodstuff applicable to products placed on the market (Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs).

Food category: The surveyed food.

- **Fish food category** – the survey specifications defined a particular subset of processed fishery products that should be sampled, specifically RTE fish which were hot smoked or cold smoked or gravad, were not frozen, and were vacuum or modified atmosphere packaged. Therefore, any reference to the food category fish in this report refers to that subset of processed fishery products that were sampled in this survey.

- **Meat products food category** – the survey specifications defined a particular subset of meat products that should be sampled, specifically those ready-to eat meat products which had been subjected to heat treatment, and were then vacuum or modified atmosphere packaged. This category includes in particular cold, cooked meat products (meat products typically made with whole or large parts of anatomical or reformed structures, e.g. cooked sliced ham and cooked chicken fillet), sausages, and pâtés. The category does not include meat products dried after heat treatment, meat products heat treated in an impermeable package which are not handled thereafter, and fermented meat products. Therefore, any reference to the food category of meat products in this report refers to that subset of the wider meat product definition which was sampled in this survey.

  Cheese food category – the survey specifications defined a particular subset of cheeses to be sampled, specifically RTE soft or semi-soft cheeses, excluding fresh cheeses. This category includes smear-ripened, mould-ripened, brine-matured or otherwise ripened, cheese made from raw, thermised or pasteurised milk of any animal species. The cheese could be packaged, or unpackaged at retail but packaged at the point of sale for the consumer. Therefore, any reference

[7] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, 30.4.2004, p.1-54.
[8] Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. OJ L 139, 30.4.2004, p.55-205.

to the food category cheese in this report means that subset of cheeses that were sampled in this survey.

Gravad fish: Fish that have been cured in salt and sugar without thermal treatment.
***Listeria monocytogenes* positive food** – a food in which *L. monocytogenes* is isolated by culture techniques from a sample taken out of it.

Meat products: Processed products resulting from the processing of meat or from the further processing of such processed products, so that the cut surface shows that the product no longer has the characteristics of fresh meat (Regulation (EC) No 853/2004 laying down specific hygiene rules for food of animal origin).

Microbiological criterion: Criterion defining the acceptability of a product, a batch of foodstuffs or a process, based on the absence, presence or number of microorganisms, and/or on the quantity of their toxins/metabolites, per unit(s) of mass, volume, area or batch (Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs).

Modified atmosphere packaging: Removal of air from a food package and replacement with a strictly controlled gaseous mixture of carbon dioxide, oxygen, and/or nitrogen, and then hermetically sealed.

Packaged food: A food that has its entire surface covered in order to prevent direct contact of the food with the environment. This would include impermeable wrapping such as hermetically sealed plastic, and also include permeable wrapping, such as muslin-wrapped cheese.

Preservatives: Substances which prolong the shelf-life of foods by protecting them against deterioration caused by microorganisms and/or which protect against growth of pathogenic micro-organisms (Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives).[9]

Processing: Any action that substantially alters the initial product, including heating, smoking, curing, maturing, drying, marinating, extraction, extrusion or a combination of those processes (Regulation (EC) No 852/2004 on the hygiene of foodstuffs).

Ready-to-eat (RTE) food: Food intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to acceptable level microorganisms of concern (Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs).

Remaining shelf-life: In this survey, it was not mandatory to report the production date of the surveyed foods, which is the date that correlates to the start of the shelf-life, was not mandatory to be reported. This implies that the true shelf-life period of the sampled foods is not always known. The smoked and gravad fish samples were analysed at the time of sample collection and at the end of shelf-life (two different packages from the batch were analysed at each time point), however, as the time of sample collection is a random time point within the shelf-life of the smoked or gravad fish, the time difference between the time of sampling and the end of shelf-life can only be referred to as the 'remaining shelf-life' but not be judged in relation to the overall shelf-life.

Retail: The handling and/or processing of food and its storage at the point of sale or delivery to the final consumer (Regulation (EC) No 178/2002 of the European Parliament and of the Council laying down the general principles and requirements of food law, establishing the European Food Safety

[9] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives (Text with EEA relevance). OJ L 354, 31.12.2008, p.16-33.

Authority and laying down procedures in matters of food safety).[10] In this survey retail covered only shops, supermarkets and other similar outlets that sell directly to the final consumer. It did not include distribution terminals or centres, catering operations, institutional catering, factory canteens, restaurants and other similar food service operations and wholesale outlets.

Shelf-life: The period from manufacture to the "*Use by*" or the minimum durability date (Directive 2000/13/EC of the European Parliament and of the Council on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs).[11]

Smoked fish: Fish cured by smoking. It is normal for fish smoking procedures to include addition of salt.

- **Cold smoked fish** – fish which has been smoked at a time-temperature combination not sufficient to coagulate muscle proteins.
- **Hot smoked fish** – fish which has been smoked at a time-temperature combination sufficient to coagulate the muscle proteins.

Vacuum packaging: Evacuation of air from a food package that is then hermetically sealed.

3. Objectives

The 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.

Specific objectives were the following:

- estimation of the EU prevalence of *L. monocytogenes* in the surveyed RTE foods,
- analysis of the qualitative and quantitative survey test results,
- 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.

This Part A report includes the analyses of the prevalence of *L. monocytogenes* and of the qualitative and quantitative survey test results. The analyses of factors related to the prevalence of contaminated foods, as well as the development of predictive models for the microbial growth of *L. monocytogenes* under various storage conditions, and for compliance with *L. monocytogenes* food safety criteria in foods, will be provided in the Part B report, which will be published at a later date.

[10] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1-24.

[11] Directive 2000/13/EC of the European Parliament and of the Council of 20 March 2000 on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs. OJ L 109, 6.5.2000, p. 29-42.

4. 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.

4.1. Survey design

As mentioned 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), the prevalence of contaminated foodstuffs to be investigated was assumed to be rare (below 0.1 %) for the purpose of the survey sample size estimation. Consequently, general sample size calculations were considered to be less valid and more appropriate methodology, for example risk-based sampling, i.e. sampling of at risk foods, such as the surveyed fish, meat products and cheese food categories, underpinned this EU level survey. Moreover, the sample size input criteria (an expected prevalence of 5 %, an accuracy of 1.5 % and a desired confidence level of 95 %) led to the number of samples to be taken being then multiplied by three, giving 3 020 samples to be taken for every specified food category, per analysis stage (analysing at time of sampling or at end of the shelf-life), at the EU level. The multiplication by 3 was used because of an absence of knowledge on any EU specific survey design effect.

Next a proportionate stratified sampling scheme was followed to allocate this number of samples to the MSs approximately according to the size of their human population. The reason for proposing an allocation scheme based on MSs' human population is that reliable and specific food marketing data were not available for all MSs. The number of samples to be taken per RTE food category in each MS was set out in Annex II of the Commission Decision 2010/678/EU. At 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 MSs:

(a) For all samples: Country, Code of the town, Code of the retail outlet, Type of retail outlet, Date of sampling, Type of sample (smoked or gravad fish, heat-treated meat product, or soft/semi-soft cheese), 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, *L. monocytogenes* quantification result at the end of the shelf-life,

L. 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 packaged heat-treated meat products: 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 packaged hot or cold smoked or gravad fish samples: 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, *L. monocytogenes* quantification on the arrival at the laboratory, *L. 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 soft or semi-soft cheese samples: 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).

For more details see Appendix A.

4.2. Laboratory analysis

The procedures for laboratory analysis of the samples are described in Commission Decision 2010/678/EU. Analyses of *L. monocytogenes*, and of pH and water activity were performed by the National Reference Laboratory in each MS, or other laboratories accredited for those analyses and designated by the Competent Authority.

All samples received were examined to ensure that the packaging used for transportation was intact before storing. Samples received at a temperature higher than 8 °C were rejected, unless the temperature at retail was higher than 8 °C.

One of the two samples of packaged smoked and gravad fish was analysed within 24 hours of the time of arrival at the laboratory. The second sample was kept refrigerated until the end of its shelf-life.

All soft and semi-soft cheeses and packaged heat-treated meat products were kept refrigerated until the end of their shelf-life.

Either the entire product, or a representative test portion of 100 to 150 g, was taken to the initial dilution. Food was sampled to include surfaces reflecting the proportion that would be consumed (such as 20 % rind/surface and 80 % inside). When a packaged product was sliced, the respective sample was taken from more than one slice of the product. The test portion was cut into small pieces and placed into a stomacher bag, using a sterile instrument and an aseptic technique, and then homogenised for one minute. From that mixture, a test portion of 10 g was taken for enumeration and a test portion of 25 g was taken for detection.

Detection and enumeration analyses of *L. monocytogenes* were performed in accordance with the following:

(a) for smoked and gravad fish samples two sets of analyses were carried out:

(i) immediately after sample collection at retail level; and

(ii) at the end of shelf-life;

(b) for soft and semi-soft cheese samples and heat-treated meat product samples the analyses were carried out only at the end of shelf-life.

Detection of *L. monocytogenes* was performed according to EN ISO 11290-1:1996[12] amended in 2004 (EN ISO 11290-1:1996/A1:2004).[13]

The enumeration of *L. monocytogenes* was performed according to EN ISO 11290-2:1998[14] and its modification EN ISO 11290-2:1998/A1:2004.[15]

More details regarding the used survey test, both detection and enumeration, can be found in Appendix B.

For fish samples tested at sampling, the measurement of the pH of the sample was performed according to EN ISO 2917:1999,[16] while the measurement of the water activity of the sample was performed according to EN ISO 21807:2004.[17]

4.3. Data validation and cleaning

MSs submitted data to the EC. Then a set of data exclusion criteria (Appendix C) was used by the EC to identify and exclude non-valid and non-plausible information in the dataset submitted by MSs. MSs corrected the excluded data. The cleaned, validated dataset was provided to EFSA by the European Commission on 21 August 2012. EFSA implemented an additional, detailed contents-level validation, in cooperation with the participating countries. Both validations resulted overall in a marginal number of samples being finally excluded. The reasons for excluding samples, in accordance with exclusion criteria, could not be exhaustively addressed because relevant information was not fully available in some cases. The final validated dataset included information on a total of 13 088 samples, sampled from 3 632 retail outlets in 26 MSs 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 for analysis. This validated dataset formed the basis for all subsequent analyses.

4.4. Statistical analysis

4.4.1. Descriptive analysis

A comparison was made of the survey protocol and the collected samples, in terms of sample size using a frequency table.

4.4.1.1. *Listeria monocytogenes* enumeration results

L. monocytogenes enumeration results from all MSs and Norway have been used for the presentation and analysis of the enumeration test counts. The distribution frequency of the enumeration test results

[12] EN ISO 11290-1:1996. Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1: Detection method (ISO 11290-1:1996).
[13] EN ISO 11290-1:1996/A1:2004. Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1: Detection method - Amendment 1: Modification of the isolation media and the haemolysis test, and inclusion of precision data (ISO 11290-1:1996/AM1:2004).
[14] EN ISO 11290-2:1998. Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 2: Enumeration method (ISO 11290-2:1998).
[15] EN ISO 11290-2:1998/A1: 2004. Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 2: Enumeration method – Amendment 1: Modification of the enumeration medium (ISO 11290-2:1998/AM1:2004).
[16] EN ISO 2917:1999. Meat and meat products — Measurement of pH — Reference method.
[17] EN ISO 21807:2004. Microbiology of food and animal feeding stuffs — Determination of water activity.

(together with associated proportions) has been tabulated for the fish samples at the end of shelf-life and at the time of sample collection as well as for the meat and cheese samples. The classes that were used for this tabulation were as follows: < 10 (colony forming units) cfu/g; 10-39 cfu/g; 40-100 cfu/g; > 100-1 000 cfu/g; > 1 000-10 000 cfu/g; > 10 000-100 000 and > 100 000 cfu/g. Measures of central tendency (geometric mean, median and mode) have been produced considering the samples that had a *L. monocytogenes* count of at least 10 cfu/g. While quantitative microbiological analysis was performed for every sample thus producing both a quantitative and qualitative result for each sample, the majority of samples indicated a result < 10 cfu/g on this analysis. The resultant skewed dataset does not easily lend itself to simple descriptions, with for example an arithmetic mean being somewhat over-representative of the small number of high and very high results. For fish samples, results are also presented separately for hot smoked, cold smoked, unknown smoked and gravad fish.

Additionally, box plots of the $\log_{10}$-transformed counts are provided for the fish samples at the time of sampling and at the end of shelf-life.

4.4.2. Estimates of prevalence of *Listeria monocytogenes* at EU level

The surveyed food data analysed originated from a complex survey design and two aspects had to be considered for the prevalence estimation. First, data were collected following a hierarchical approach using a multistage cluster sampling design, considering three levels of subsequent selection and sampling: major cities/towns; retail outlets; and the food product (batch, applicable only for fish samples). It is expected that batches within an outlet are more alike than batches from different outlets (clustering issue). Second, sample size did not accurately reflect a country's human population, thus resulting in disproportionate sampling for the EU estimation of prevalence, necessitating consideration of whether it would be necessary for subsequent weighting for the latter analysis.

The prevalence of *L. monocytogenes*-contaminated food categories was estimated at EU level for the following surveys and outcomes;

- *L. monocytogenes* in fish, at end of shelf-life, and at time of sample collection;
- *L. monocytogenes* in meat products at the end of shelf-life;
- *L. monocytogenes* in cheeses at the end of shelf-life.

Also, separate estimates are produced for hot smoked, cold smoked, gravad fish and unknown smoked fish.

Estimates were produced for two different parameters:

a. 'Prevalence': this parameter was based on combined detection and enumeration methods results. A food sample was considered positive if *L. monocytogenes* was detected by at least one of either the detection or the enumeration method, (i.e. a sample was regarded as positive when either the detection test result was positive and/or the enumeration test result was positive, i.e. having a count of at least 10 cfu/g). As the potential for false-positive results is low with both methods, and non-homogenous bacterial distribution might well account for discordance particularly for low counts, any positive result was regarded as indicating that sample was positive.

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

Several modelling approaches were used to estimate the two parameters of interest. The result was that the most appropriate ones were logistic regression models using generalised estimating equations (GEE) methodology to empirically correct the standard errors for the possible presence of correlation within clusters. Consequently, confidence intervals (CIs) of prevalence estimates were wider than

those that would have been obtained by using ordinary logistic regression not taking into account within-cluster correlation. An independence correlation structure was used, a plausible choice assuming that there was no logical ordering of batches within an outlet. The independence correlation structure was preferred over an exchangeable structure; because the corresponding estimates for the prevalence/proportion are identical to the sample proportions (and hence easy to reconstruct). The estimates are consistent and the GEE methodology (sandwich variance estimator) provides corrected standard errors. For the exchangeable structure, estimates for the prevalence/proportion can differ from the sample proportions. If the exchangeable structure is correct, these estimates are expected to be somewhat more efficient (smaller standard errors). But the results indicated that there is a concern about the correctness of the exchangeable structure, therefore, the independence correlation structure was used for the main inferences. Moreover, the use of weights in the estimation in order to correct for overrepresentation or under-representation of certain MSs was investigated. The result was that it was appropriate to report unweighted results, which has been done in the present report.

A detailed description on statistical models and weighting is given in the External Report (Rakhmawati et al., 2013).

Cross-tabulations between each of the two parameters of interest at time of sampling and at the end of shelf-life for the surveyed fish samples are presented. Furthermore, simple cross-tabulations between detection and enumeration test results at the end of shelf-life for all surveyed food samples and also at the time of sample collection for the surveyed fish samples are also presented in Appendix G.

5. Results

5.1. Sample Summary and sample-protocol comparison

A table describing the planned and achieved sample size for each country participating in the survey can be found in Appendix D. Some MSs submitted fewer samples than planned, while some MSs provided data for substantially more samples than expected. Overall, there was good compliance with the planned sample size. Portugal did not report results for this survey.

As mentioned above, the final validated dataset included information on 13 088 samples, sampled from 3 632 retail outlets in 26 MSs and Norway: 3 053 smoked or gravad fish samples analysed at sampling and 3 053 smoked or gravad fish samples analysed 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. This validated dataset formed the basis for all subsequent analyses.

5.2. *Listeria monocytogenes* survey results in the fish food category

5.2.1. Descriptions of the samples

Details on the sample characteristics of the surveyed fish as well as the storage temperatures at retail and at the laboratory up to the end of shelf-life can be found in Appendix E. In order to summarise the reported information on the species of fish it was necessary to classify the data in the following specific classes of fish: salmon, herring, mackerel, mixed fish and other fish (for fish species not already mentioned above). As can be seen in Table 1, the surveyed fish were predominantly salmon (60.9 %). It is interesting to note that 10.7 % of the sampled fish products contained more than one fish species (mixed fish).

Table 1: Distribution of fish species sampled for the *L. monocytogenes* baseline survey in the EU,[(a)] 2010-2011

Fish species	Hot smoked	%	Cold smoked	%	Gravad	%	Unknown smoked	%	Total	%
Salmon	193	36.1	455	71.1	164	64.8	1 047	64.4	1 859	60.9
Herring	25	4.7	28	4.4	29	11.5	101	6.2	183	6.0
Mackerel	120	22.4	63	9.8	5	2.0	222	13.7	410	13.4
Mixed Fish	128	23.9	57	8.9	17	6.7	124	7.6	326	10.7
Other Fish	69	12.9	37	5.8	38	15.0	131	8.1	275	9.0
Total	535		640		253		1 625		3 053	

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

Ninety-eight percent of the surveyed fish samples originated from a 'Supermarket or small shop', while more than half (59.8 %) were vacuum packaged (Table 5, Appendix E). The average pH of the samples on arrival at the laboratory was 6.03, while the average water activity (a_w) was 0.96 (Table 8, Appendix E). The distributions of pH and a_w are shown in Figures 6 and 7 in Appendix E.

In order to provide a general description of the preservatives and acidity regulators reported for the surveyed fish samples (as indicated on the label), the following three classes were used: samples without any reported preservatives and/or acidity regulators, samples with one reported preservative or acidity regulator, and samples with two or more preservatives and/or acidity regulators. The following reported additives were not counted when assigning each fish sample into one of the above classes: sodium chloride, potassium chloride, sugar, smoke, herbs and spices. For more details on this classification, see Appendix F. Based on these criteria, a total of 138 surveyed fish samples (4.5 %) were reported as containing at least one preservative and/or acidity regulator (see Table 9 in Appendix E).

The mean storage temperature at retail for the surveyed fish samples was 3.5 °C with a standard deviation of 1.8 °C (Table 10, Appendix E). In addition, among samples for which this information was reported, 98.0 % were suitable for human consumption at the end of shelf-life on the basis of visual and smell (olfactory) evaluation by the laboratory analyst (Table 3, Appendix E).

Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs specifies that 'Products with pH ≤ 4.4 or $a_w \leq 0.92$, products with pH ≤ 5.0 and $a_w \leq 0.94$, products with a shelf-life of less than five days' are automatically considered as belonging to the category of RTE foods unable to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes'. In the current baseline survey, there were 210 packaged fish samples (batches), which fulfilled at least one of the above pH and/or a_w criteria. These batches originated from almost all participating countries, 24 MSs and Norway. Sixty-six of these samples were salmon, 40 herring, 41 mackerel, 22 mixed fish and 41 other fish. Among those, there were 25 batches that had a positive result, either with detection and/or enumeration at sampling and/or at the end of shelf-life. The inclusion of these products in the surveyed fish samples reflects the challenge faced at an official control level in selecting food products which might support the growth of *L. monocytogenes*, in advance of laboratory analysis to determine a_w and pH. Furthermore, these products would seem to represent some of the types of smoked or gravad RTE fishery products for sale in the EU under refrigeration. Since the presence of *L. monocytogenes* in these samples was similar to that in the remaining fish samples in the survey, these samples were not excluded from the estimations presented in this report, i.e. they were included within overall analyses, not as a specific subset. It also has to be noted that other fish samples in this baseline survey may also not support growth, for example, if they contain inhibitory concentrations of added preservatives and/or acidity regulators, however, this cannot be conclusively addressed, as there is no information in the dataset concerning the concentration of such additives in the fish samples.

Figures 8 and 9 in Appendix E summarize the remaining shelf-life of the surveyed fish samples. This is the difference, in days, between the date of sampling and the 'Use by' date of the sampled product. The mean remaining shelf-life of the surveyed fish samples was 22.7 days, however, some important variability along with many extreme values was observed (standard deviation 36.7 days and range from 1 to 519 days).

5.2.2. Prevalence of *Listeria monocytogenes*-contaminated fish samples

The food category of RTE packaged smoked or gravad fish was assessed twice in this study, at two different time points during the shelf-life. An EU prevalence of 10.4 % and 10.3 % was found, respectively, at the time of sampling and at the end of shelf-life (Tables 2 and 3).

The differences in observed prevalence of *L. monocytogenes*–contaminated fish categories when comparing the time of sampling to end of shelf-life, as well as amongst fish subcategories, will be investigated further in the Report Part B taking account of the important variability of the remaining shelf-life of the surveyed fish samples.

Table 2: Prevalence (%) of *L. monocytogenes*-contaminated packaged smoked or gravad fish samples, at sampling, in the EU,[(a)] 2010-2011

Subtype of the fish product	Total No of samples	No of positive of samples	Prevalence (%)	95 % CI
Cold smoked fish	599	104	17.4	14.2 – 21.1
Hot smoked fish	525	33	6.3	4.4 – 8.9
Unknown smoked fish	1 625	143	8.8	7.3 – 10.5
Gravad fish	245	30	12.2	8.7 – 17.0
EU	**2 994**	**310**	**10.4**	**9.1 – 11.7**

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. Norway is not included in the EU prevalence estimation analysis.

Table 3: Prevalence (%) of *L. monocytogenes*-contaminated packaged smoked or gravad fish samples, at end-of-shelf-life, in the EU,[(a)] 2010-2011

Subtype of the fish product	Total No of samples	No of positive samples	Prevalence (%)	95 % CI
Cold smoked fish	599	96	16.0	13.2 – 19.3
Hot smoked fish	525	35	6.7	4.7 – 9.3
Unknown smoked fish	1 625	148	9.1	7.6 – 10.9
Gravad fish	245	30	12.2	8.6 – 17.1
EU	**2 994**	**309**	**10.3**	**9.1 – 11.6**

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. Norway is not included in the EU prevalence estimation analysis.

The proportion (and number) of fish samples with a *L. monocytogenes* count exceeding the level of 100 cfu/g was 1 % (29 samples) at time of sampling and 1.7 % (52 samples) at the end of shelf-life (Tables 4 and 5).

Table 4: Proportion (%) of packaged hot or cold smoked or gravad fish samples exceeding the level of 100 cfu/g at sampling, in the EU,[(a)] 2010-2011

Subtype of the fish product	Total No of samples	No of samples > 100 cfu/g	Proportion of samples > 100 cfu/g (%)	95 % CI
Cold smoked fish	599	10	1.7	0.9 – 3.2
Hot smoked fish	525	7	1.3	0.6 – 2.8
Unknown smoked fish	1 625	10	0.6	0.3 – 1.2
Gravad fish	245	2	0.8	0.2 – 3.2
EU	**2 994**	**29**	**1.0**	**0.7 – 1.4**

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

Table 5: Proportion (%) of packaged hot or cold smoked or gravad fish samples exceeding the level of 100 cfu/g at end of shelf-life, in the EU,[(a)] 2010-2011

Subtype of the fish product	Total No of samples	No of samples > 100 cfu/g	Proportion of samples > 100 cfu/g (%)	95 % CI
Cold smoked fish	599	12	2.0	1.1 – 3.6
Hot smoked fish	525	9	1.7	0.9 – 3.3
Unknown smoked fish	1 625	29	1.8	1.2 – 2.6
Gravad fish	245	2	0.8	0.2 – 3.2
EU	**2 994**	**52**	**1.7**	**1.3 – 2.3**

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

5.2.2.1. Description of fish samples with a count of *Listeria monocytogenes* exceeding the level of 100 cfu/g

Fourteen batches of fish had samples with counts exceeding the level of 100 cfu/g at both testing times and 67 batches of fish had counts that exceeded 100 cfu/g in at least at one of the two testing times. These batches originated from 17 MSs and were 16 batches of cold smoked fish, 15 of hot smoked, 32 of unknown smoked and four of gravad fish. Moreover, 48 were salmon, five mackerel, one herring, four mixed fish and nine other fish. Their pH at time of sampling ranged from 5.18 to 6.7 and their water activity from 0.91 to 0.99, nine were not sliced and 58 were sliced, while nine were packed in modified atmosphere, three in normal atmosphere, 54 in vacuum and one in 'other'. In addition, in 64 batches there were no added preservatives nor acidity regulators, while in three batches there were two or more preservatives and/or acidity regulators added. It is interesting to note that the proportion of batches without any preservatives or acidity regulators added among these samples (95.5 %) is not dissimilar to the respective proportion in the entire surveyed fish dataset. Finally, for 60 out of those 67 batches information was reported on whether they were suitable for human consumption at the end of shelf-life, on the basis of visual and smell (olfactory) evaluation. For 18 of those batches it was specified that they were not suitable for human consumption at the end of shelf-life, on the basis of visual and smell (olfactory) evaluation. The Report Part B will report with more details on the factors associated with the prevalence of *L. monocytogenes*-contaminated foods.

5.2.3. *Listeria monocytogenes* enumeration results in fish

L. monocytogenes counts of < 10 cfu/g of food correspond to the absence of *L. monocytogenes* detection in the enumeration method, i.e. no *L. monocytogenes* colonies developing in the incubated selective solid media inoculated with 1 millilitre of the initial food sample suspension. The reason for grouping counts between 10 and 39 cfu/g into one separate category was that counts below 40 are considered to be of too low a precision and are normally reported as 'presence of *L. monocytogenes*', in agreement with ISO 7218:2007.[18]

5.2.3.1. Overall results for the surveyed fish

At the EU level, the percentages of fish samples, at sampling, with enumeration results (cfu/g of food) below 10, between 10-39, between 40-100, above 100-1 000, above 1 000-10 000, above 10 000-100 000 and above 100 000 were 97.8 %, 0.6 %, 0.6 %, 0.7 %, 0.2 %, 0.1 % and 0.1 %, respectively (Table 6). At the end of shelf-life, these percentages were 96.8 %, 0.9 %, 0.6 %, 0.9 %, 0.4 %, 0.3 % and 0.1 %, respectively (Table 7). Norway's data are included in these results.

In general terms, the data suggest a subtle increase in the quantitative load of *L. monocytogenes* in the fish category, when comparing the time of sampling to the end of shelf-life, because the overall dataset showed that 66 samples (2.2 %) at time of sampling and 99 samples (3.2 %) at the end of shelf-life had a *L. monocytogenes* count of at least 10 cfu/g, out of the 3 053 samples that were examined at each time point (Table 8). However, when considered as paired samples it has to be noted that not all 66 samples with a count of at least 10 cfu/g at time of sampling had a count of at least 10 cfu/g at the end of shelf life. Some comparative statistics are further shown in Table 8, for example the median count of cfu/g of food was 70 at time of sampling (for the 66 samples with a count of at least 10 cfu/g) and 100 at the end of shelf-life (for the 99 samples with a count of at least 10 cfu/g). A box plot of the $\log_{10}$-transformed *L. monocytogenes* counts for the samples with a count of 10 cfu/g or above is shown in Figure 1 for the time of sampling and in Figure 2 for the end of shelf-life.

The differences in quantitative loads for *L. monocytogenes* in fish categories when comparing the time of sampling to the end of shelf-life, as well as among sampled fish subcategories, will be investigated further in the Report Part B that will also report on the factors associated with the prevalence of contaminated foods.

Table 6: Distribution frequency of *L. monocytogenes* counts (cfu/g) present in packaged hot or cold smoked or gravad fish samples at sampling, in the EU,[(a)] 2010-2011

	***L. monocytogenes* count (cfu/g)**							
	< 10[(b)]	**10-39**	**40-100**	**> 100-1 000**	**> 1 000-10 000**	**> 10 000-100 000**	**> 100 000**	**Total**
Total No of samples	2 987	18	19	20	5	2	2	3 053
Proportion of samples (%)	97.8	0.6	0.6	0.7	0.2	0.1	0.1	
Proportion of samples amongst 66 samples with at least 10 cfu/g	-[(c)]	27.3	28.8	30.3	7.6	3.0	3.0	

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

[18] ISO 7218:2007. Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations.

(b): This category includes the samples with results below the limit of detection of the enumeration method. It also includes samples not contaminated with *L. monocytogenes*.
(c): not applicable.

Table 7: Distribution frequency of *L. monocytogenes* counts (cfu/g) present in packaged hot or cold smoked or gravad fish samples at end of shelf-life, in the EU,[(a)] 2010-2011

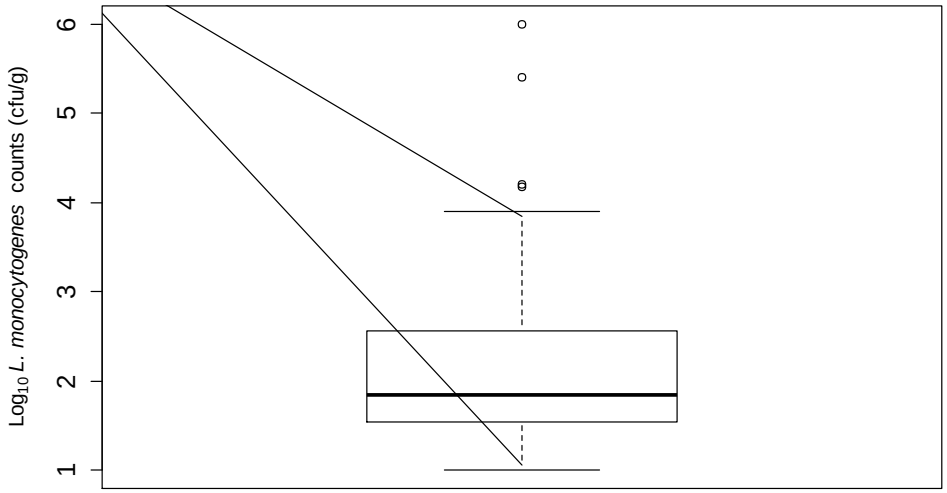
	***L. monocytogenes* count (cfu/g)**							
	< 10[(b)]	**10-39**	**40-100**	**> 100-1 000**	**> 1 000-10 000**	**> 10 000-100 000**	**> 100 000**	**Total**
Total No of samples	2 954	28	19	27	12	10	3	3 053
Proportion of samples (%)	96.8	0.9	0.6	0.9	0.4	0.3	0.1	
Proportion of samples amongst 99 samples with at least 10 cfu/g	-[(c)]	28.3	19.2	27.3	12.1	10.1	3.0	

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.
(b): This category includes the samples with results below the limit of detection of the enumeration method. It also includes samples not contaminated with *L. monocytogenes*.
(c): not applicable.

Table 8: Summary statistics of *L. monocytogenes* counts (cfu/g) present in packaged hot or cold smoked or gravad fish samples at sampling and at end of shelf-life, for samples with a *L. monocytogenes* count of at least 10 cfu/g, in the EU,[(a)] 2010-2011

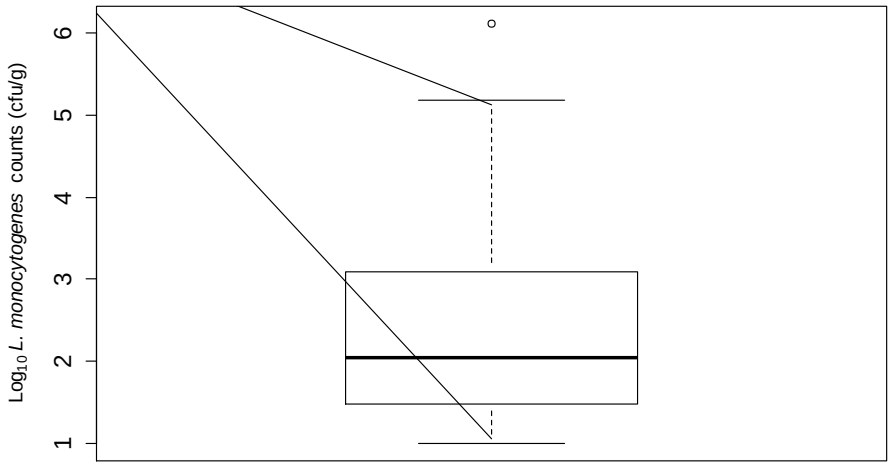
Summary statistic	**At sampling**	**At end of shelf-life**
Total No of samples	66	99
Geometric mean[(b)]	139	248
Median[(c)]	70	110
Mode[(d)]	10	40

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.
(b): Geometric mean is a type of mean or average which is defined as the n^{th} root (where n is number) of the product of the numbers.
(c): Median is the numerical value separating the higher half of the data. For an odd number of observations, the median is the central point of the data. For an even number of observations, there is no single middle value; the median is defined as the mean of the two middle values.
(d): The mode is defined as the value that appears most often in a set of data.



(a): The lower whisker represents the lowest value, the bottom of the box represents the first quartile of the distribution and the top the third quartile, whereas the bar inside the box represents the median. The upper whisker represents the maximum value or 1.5 times the difference between the third and the first quartile (interquartile range). Small circular symbols indicate extreme values, with a value larger than the upper whisker.
(b): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.

Figure 1: Box plot[(a)] of the $\log_{10}$-transformed *L. monocytogenes* counts (cfu/g) present in packaged hot or cold smoked or gravad fish samples at sampling for the 66 samples with a count of 10 cfu/g or above, in the EU,[(b)] 2010-2011



(a): The lower whisker represents the lowest value, the bottom of the box represents the first quartile of the distribution and the top the third quartile, whereas the bar inside the box represents the median. The upper whisker represents the maximum value or 1.5 times the difference between the third and the first quartile (interquartile range). Small circular symbols indicate extreme values, with a value larger than the upper whisker.
(b): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.

Figure 2: Box plot[(a)] of the $\log_{10}$-transformed *L. monocytogenes* counts (cfu/g) present in packaged hot or cold smoked or gravad fish samples at the end of shelf-life for the 99 samples with a count of 10 cfu/g or above, in the EU,[(b)] 2010-2011

5.2.3.2. Results for the surveyed fish subcategories; hot smoked, cold smoked, unknown smoked and gravad fish

Table 9: Distribution frequency of *L. monocytogenes* counts (cfu/g) present in packaged hot or cold smoked or gravad fish samples at sampling, in the EU,[(a)] 2010-2011

		***L. monocytogenes* count (cfu/g)**							
		< 10[(b)]	**10-39**	**40-100**	**> 100-1 000**	**> 1 000-10 000**	**> 10 000-100 000**	**> 100 000**	**Total**
Hot smoked fish	Total No of samples	523	2	3	4	1	1	1	535
	Proportion of samples (%)	97.8	0.4	0.6	0.7	0.2	0.2	0.2	
	Proportion of samples among those with at least 10 cfu/g	-[(c)]	16.7	25.0	33.3	8.3	8.3	8.3	
Cold smoked fish	Total No of samples	615	8	7	7	2	1	0	640
	Proportion of samples (%)	96.1	1.3	1.1	1.1	0.3	0.2	0	
	Proportion of samples among those with at least 10 cfu/g	-	32.0	28.0	28.0	8.0	4.0	0	
Gravad fish	Total No of samples	249	2	0	2	0	0	0	253
	Proportion of samples (%)	98.4	0.8	0	0.8	0	0	0	
	Proportion of samples among those with at least 10 cfu/g	-	50.0	0	50.0	0	0	0	
Unknown smoked fish	Total No of samples	1 600	6	9	7	2	0	1	1 625
	Proportion of samples (%)	98.5	0.4	0.6	0.4	0.1	0	0.1	
	Proportion of samples among those with at least 10 cfu/g	-	24.0	36.0	28.0	8.0	0	4.0	

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.
(b): This category includes the samples with results below the limit of detection of the enumeration method. It also includes samples not contaminated with *L. monocytogenes*.
(c): not applicable.

Table 10: Summary statistics of *L. monocytogenes* counts (cfu/g) present in packaged hot or cold smoked or gravad fish samples at sampling, for the 66 samples with a *L. monocytogenes* count of at least 10 cfu/g, in the EU,[(a)] 2010-2011

Summary statistic	Hot smoked fish	Cold smoked fish	Gravad fish	Unknown smoked fish
Total No of samples	12	25	4	25
Geometric mean[(b)]	319	103	68	140
Median[(c)]	150	50	160	48
Mode[(d)]	-[(e)]	10	-	40

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.
(b): Geometric mean is a type of mean or average which is defined as the n^{th} root (where n is the count of numbers) of the product of the numbers.
(c): Median is the numerical value separating the higher half of the data. For an odd number of observations, the median is the central point of the data. For an even number of observations, there is no single middle value; the median is defined to be the mean of the two middle values.
(d): The mode is defined as the value that appears most often in a set of data.
(e): The mode does not exist, since there is no unique count value that appears with a higher frequency than the rest of count values.

Table 11: Distribution frequency of *L. monocytogenes* counts (cfu/g) present in packaged hot or cold smoked or gravad fish samples at the end of shelf-life, in the EU,[(a)] 2010-2011

		***L. monocytogenes* count (cfu/g)**							
		< 10[(b)]	**10-39**	**40-100**	**> 100-1 000**	**> 1 000-10 000**	**> 10 000-100 000**	**> 100 000**	**Total**
Hot smoked fish	Total No of samples	519	2	5	3	4	2	0	535
	Proportion of samples (%)	97.0	0.4	0.9	0.6	0.7	0.4	0	
	Proportion of samples among those with at least 10 cfu/g	-[(c)]	12.5	31.3	18.8	25.0	12.5	0	
Cold smoked fish	Total No of samples	614	12	2	6	2	4	0	640
	Proportion of samples (%)	95.9	1.9	0.3	0.9	0.3	0.6	0	
	Proportion of samples among those with at least 10 cfu/g	-	46.2	7.7	23.1	7.7	15.4	0	
Gravad fish	Total No of samples	244	4	3	1	1	0	0	253
	Proportion of samples (%)	96.4	1.6	1.2	0.4	0.4	0	0	
	Proportion of samples among those with at least 10 cfu/g	-	44.4	33.3	11.1	11.1	0	0	
Unknown smoked fish	Total No of samples	1 577	10	9	17	5	4	3	1 625
	Proportion of samples (%)	97.0	0.6	0.6	1.0	0.3	0.2	0.2	
	Proportion of samples among those with at least 10 cfu/g	-	20.8	18.8	35.4	10.4	8.3	6.3	

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.
(b): This category includes the samples with results below the limit of detection of the enumeration method. It also includes samples not contaminated with *L. monocytogenes*.
(c): not applicable.

Table 12: Summary statistics of *L. monocytogenes* counts (cfu/g) present in the subtypes of the fish product samples at the end of shelf-life, for the 99 samples with a *L. monocytogenes* count of at least 10 cfu/g, in the EU,[(a)] 2010-2011

Summary statistic	Hot smoked fish	Cold smoked fish	Gravad fish	Unknown smoked fish
Total No of samples	16	26	9	48
Geometric mean[(b)]	409	163	57	346
Median[(c)]	170	55	40	145
Mode[(d)]	6 300	30	-[(e)]	40

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.
(b): Geometric mean is a type of mean or average which is defined as the n^{th} root (where n is number) of the product of the numbers.
(c): Median is the numerical value separating the higher half of the data. For an odd number of observations, the median is the central point of the data. For an even number of observations, there is no single middle value; the median is defined as the mean of the two middle values.
(d): The mode is defined as the value that appears most often in a set of data.
(e): The mode does not exist, since there is no unique count value that appears with a higher frequency than the rest of count values.

A cross-classification of test results for detection and enumeration tests for packaged smoked or gravad fish samples can be found in Appendix G.

5.2.4. Comparison of test results for fish at end of shelf-life and at time of sample collection

Each batch of fishery products had two samples taken and analysed at different points in the shelf-life. Table 13 shows the cross-classification of surveyed fish samples at time of sampling and at the end of shelf-life, based on the same definition of a contaminated sample as the one used for the EU *L. monocytogenes* prevalence estimations. In 91.1 % of batches (2 727 out of 2 994) there was agreement between analysis at time of sampling and analysis at end of shelf-life. There was a high proportion of negative results at both sampling times. It is interesting to note that 43.2 % of the batches (134 batches out of 310) with the sample analysed at the time of sample collection were contaminated with *L. monocytogenes*, whereas the second sample of those batches, analysed at the end of shelf-life, was negative in both the detection and the enumeration test. Conversely, 43.0 % of the batches (133 batches out of 309) with the second sample analysed at the end of shelf-life were contaminated with *L. monocytogenes*, whereas the first sample of those batches, analysed at the time of sample collection, was negative in both detection and enumeration test.

The differences in these test results will be investigated further in the Report Part B.

Table 13: Classification of packaged hot or cold smoked or gravad fish samples based on the combined *L. monocytogenes* prevalence definition (positive with the detection test and/or with a count of at least 10 cfu/g with the enumeration test) at sampling and at the end of shelf-life, in the EU,[(a)] 2010-2011

At sampling	At the end of shelf-life		
	Negative	Positive	Total
Negative	2 551	133	2 684
Positive	134	176	310
Total	2 685	309	2 994

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

Table 14 shows the cross-classification of surveyed fish samples, based on whether they had a *L. monocytogenes* count of > 100 cfu/g, at sampling and at end of shelf-life. In 98.2 % of batches (2 941 out of 2 994) there was agreement between analysis at time of sampling and analysis at end of shelf-life. However, there was a high frequency of samples with counts less than 100 cfu/g at both sampling points. There was 51.7 % of the batches (15 batches out of 29) with the sample analysed at the time of sample collection having a count of *L. monocytogenes* exceeding the level of 100 cfu/g, whereas the second sample of those batches, analysed at the end of shelf-life, had a count of maximum 100 cfu/g. Conversely, 73.1 % of the batches (38 batches out of 52) with the second sample analysed at the end of shelf-life had a count of *L. monocytogenes* exceeding the level of 100 cfu/g, whereas the first sample of those batches, analysed at the time of sample collection, had a count of maximum 100 cfu/g.

The differences in these test results will be investigated further in the Report Part B.

Table 14: Classification of packaged hot or cold smoked or gravad fish samples based on having a *L. monocytogenes* count of above 100 cfu/g at sampling and at the end of shelf-life, in the EU,[(a)] 2010-2011

At sampling	At the end of shelf-life		
	< = 100 cfu/g	**> 100 cfu/g**	**Total**
< = 100 cfu/g	2 927	38	2 965
> 100 cfu/g	15	14	29
Total	2 942	52	2 994

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

5.3. *Listeria monocytogenes* survey results in packaged heat-treated meat products, at the end of shelf-life

5.3.1. Descriptions of the samples

Details of the sample characteristics of the surveyed meat products as well as the storage temperatures at retail and at the laboratory up to the end of shelf-life can be found in Appendix E. Most of these samples (98.2 %) were obtained from supermarkets or small shops (Table 6, Appendix E). According to the data submitted by the participating countries, at the time of sampling, there appeared to be some variation in the samples' surface temperature at retail (Figure 12, Appendix E). Nonetheless, the surface temperature recorded in the vast majority of products was 7 °C or below (mean storage temperature at retail = 3.7 °C, Table 10, Appendix E).

The majority of the heat-treated meat products represented cold, cooked meat products (72.2 %). The remaining meat products were explicitly identified and classified as either sausage (22.1 %) or pâté (5.8 %), (Table 6, Appendix E). Most of the sampled products were made of pork (72.7 %). Nonetheless, products made of mixed meat or other animal-origin meat were also included in the survey (Table 6, Appendix E). Most (85.1 %) of the sampled meat products were sold in sliced form, while more than half (56.7 %) of the sampled products were reported to be modified atmosphere packaged. A considerable proportion of products were reported as normal atmosphere (15.5 %) or vacuum packaged (25.2 %) (Table 6, Appendix E).

Upon sampling, surveyed meat products were transported to the laboratories, where they were kept refrigerated until analysis at the end of their shelf-life. The frequency distribution of recorded storage temperature of the surveyed meat products in the laboratory up to the end of shelf-life is presented in Figure 13, Appendix E. The recorded mean storage temperature at the laboratory up to the end of shelf-life was 4.5 °C. Among samples for which this information was reported, 98.6 % were suitable for human consumption at the end of shelf-life on the basis of visual and smell (olfactory) evaluation

(Table 3, Appendix E). Measurements of pH and water activity were not available for the surveyed meat samples.

5.3.2. Prevalence of *Listeria monocytogenes*-contaminated meat product samples

The EU prevalence of *L. monocytogenes*-contaminated meat products was 2.07 % (CI: 1.63 % - 2.64 %) (72 positive samples out of 3 470), at the end of shelf-life.

The proportion (and number) of meat products samples with a *L. monocytogenes* count exceeding the level of 100 cfu/g was 0.43 % (CI: 0.25 %-0.74 %) (15 samples) at the end of shelf-life.

5.3.2.1. Description of meat product samples with a count of *Listeria monocytogenes* exceeding the level of 100 cfu/g

These 15 samples originated from nine MSs, and the distribution of the animal species of the origin of the meat product for those samples was the following: eight pork; one beef; two broiler; two poultry; one turkey; and one mixed. Twelve were reported as 'cold, cooked meat product', two as 'pate' and one as 'sausage'. All, except one, were sliced meat products. Seven samples were packaged in modified atmosphere, two in normal atmosphere, five in vacuum and one in 'other'. Concerning their suitability for human consumption at the end of shelf-life on the basis of visual and smell (olfactory) evaluation, 11 samples were reported as suitable for human consumption, while this information was missing for the remaining four samples.

5.3.3. *Listeria monocytogenes* enumeration results in packaged heat-treated meat products

At EU level, the percentages of meat product samples, with enumeration results (cfu/g of food) below 10, between 10-39, between 40-100, above 100-1 000, above 1 000-10 000, above 10 000-100 000 and above 100 000 were 99.1 %, 0.3 %, 0.2 %, 0.3 %, 0.03 %, 0.1 % and 0 %, respectively (Table 15). Norway's data are included in these results.

Thirty-two samples (0.9 %) had a *L. monocytogenes* count of at least 10 cfu/g, out of the 3 530 samples that were examined (Table 15). Approximately half of these products (17 out of the 32) contained *L. monocytogenes* at levels ranging from 10 to 100 cfu/g. The levels of the pathogen exceeded 100 cfu/g in 15 meat products (0.42 %, i.e. 15 out of 3 530). In three of these meat products *L. monocytogenes* counts were in excess of 1 000 cfu/g. Some summary statistics for these 32 samples are further shown in Table 16, for example the median *L. monocytogenes* count of cfu/g of food among samples with a count of at least 10 cfu/g was 93.

Table 15: Categorised *L. monocytogenes* counts (cfu/g) present in packaged heat-treated meat product samples, at the end of shelf-life, in the EU,[(a)] 2010-2011

	***L. monocytogenes* count (cfu/g)**							
	< 10[(b)]	**10-39**	**40-100**	**> 100-1 000**	**> 1 000-10 000**	**> 10 000-100 000**	**> 100 000**	**Total**
Total No of samples	3 498	9	8	12	1	2	0	3 530
Proportion of samples (%)	99.1	0.3	0.2	0.3	0.03	0.1	0	
Proportion of samples among those with at least 10 cfu/g	-[(c)]	28.1	25.0	37.5	3.1	6.3	0	

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.
(b): This category includes the samples with results below the limit of detection of the enumeration method. It also includes samples not contaminated with *L. monocytogenes*.
(c): not applicable.

Table 16: Summary statistics of *L. monocytogenes* counts (cfu/g) present in packaged heat-treated meat product samples, at the end of shelf-life, for samples with a *L. monocytogenes* count of at least 10 cfu/g, in the EU,[(a)] 2010-2011

Summary statistic	
Total No of samples	32
Geometric mean[(b)]	160
Median[(c)]	93
Mode[(d)]	20

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.
(b): Geometric mean is a type of mean or average which is defined as the n^{th} root (where n is number) of the product of the numbers.
(c): Median is the numerical value separating the higher half of the data. For an odd number of observations, the median is the central point of the data. For an even number of observations, there is no single middle value; the median is defined as the mean of the two middle values.
(d): The mode is defined as the value that appears most often in a set of data.

A cross-classification of test results for detection and enumeration tests for packaged heat-treated meat product samples can be found in Appendix G.

5.4. *Listeria monocytogenes* survey results in soft or semi-soft cheeses, at the end of shelf-life

5.4.1. Descriptions of the samples

Details of the sample characteristics of the surveyed cheese, as well as the storage temperatures at retail and at the laboratory up to the end of shelf-life, can be found in Appendix E. Most of these samples (97.5 %) were obtained from supermarkets or small shops (Table 7, Appendix E). At the time of sampling, there appeared to be some variation in the samples' surface temperature at retail (Figure 14, Appendix E). The mean storage temperature of sampled cheeses at retail was 4.1 °C, with a standard deviation of 1.8 °C (Table 10, Appendix E).

The cheese subcategory sampled with the highest frequency was mould-ripened cheeses (35.6 %). Ripening status was not known for 27.6 % of the cheeses, whereas smear-ripened (8.4 %) and brine-matured cheeses (5.5 %) were among the least-sampled cheese subcategories (Table 7, Appendix E). Most of the sampled cheeses were made of pasteurised milk (64.8 %) and a sizeable proportion (13.8 %) were manufactured using raw milk (Table 7, Appendix E). Almost three out of four sampled cheeses were manufactured using bovine milk (72.8 %), whereas cheeses made using small-ruminants' milk (sheep and goat) accounted for approximately 10 % of the sampled products; the species-origin of milk was unknown for 15.0 % of the cheeses (Table 7, Appendix E). The sample portions used for microbiological analyses included part of the cheese rind for approximately two out of three cheese products, while in the majority of these products (61.9 %), the estimated proportion of rind accounted for 20 % or less of the respective test portion (Table 7, Appendix E).

In addition, almost two out of three (68.7 %) of the sampled cheeses were reported by the participating countries as packaged under normal atmosphere, whereas modified atmosphere and vacuum packaging accounted for 12.8 % and 8.6 % of the packaging type of cheese samples included in the survey, respectively (Table 7, Appendix E).

Upon sampling, surveyed cheese products were transported to the laboratories where they were kept refrigerated until analysis at the end of their shelf-life. The frequency distribution of recorded storage temperature of the surveyed cheese products at the laboratory up to the end of shelf-life is presented in Figure 15, Appendix E. The mean storage temperature at the laboratory up to the end of shelf-life was 5.1 °C. Based on visual and smell (olfactory) evaluation, the vast majority (91.7 %) of the surveyed cheese products were deemed to be suitable for human consumption at the end of shelf-life (Table 3, Appendix E), i.e. immediately prior to testing for *L. monocytogenes*. Measurements of pH and water activity were not available for the surveyed cheese samples.

5.4.2. Prevalence of *Listeria monocytogenes*-contaminated soft or semi-soft cheeses

The EU prevalence of *L. monocytogenes*-contaminated cheese samples was 0.47 % (CI: 0.29 %-0.77 %) (16 positive samples out of 3 393) at the end of shelf-life.

The proportion (and number) of cheese samples with a *L. monocytogenes* count exceeding the level of 100 cfu/g was 0.06 % (CI: 0.02 %-0.24 %) (2 samples out of 3 393) at the end of shelf-life.

5.4.2.1. Description of cheese samples with a count of *Listeria monocytogenes* exceeding the level of 100 cfu/g

The two cheese samples with a *L. monocytogenes* count exceeding the level of 100 cfu/g were reported by two MSs, they were both made from cow's milk, one was mould-ripened and made from pasteurized milk and the other was smear-ripened and made from raw milk. The former did not have cheese rind included in the analysis, while the latter did, with a reported estimated percentage of rind ≤ 20 %. Neither of the two samples was sliced, they were both packed in a normal atmosphere and their storage temperature at the laboratory up to the end of shelf-life was 4 °C.

5.4.3. *Listeria monocytogenes* enumeration results in soft or semi-soft cheeses

At the EU level, the percentages of cheese samples, with enumeration results (cfu/g of food) below 10, between 10-39, between 40-100, above 100-1 000, above 1 000-10 000, above 10 000-100 000 and above 100 000 were 99.9 %, 0.03 %, 0.03 %, 0 %, 0.03 %, 0.03 % and 0 %, respectively (Table 17). Norway's data are included in these results.

Four samples (0.12 %) had a *L. monocytogenes* count of at least 10 cfu/g, out of the 3 452 samples that were examined (Table 17). Two samples contained *L. monocytogenes* at levels ranging from 10 to 100 cfu/g. In the two other samples the levels of the pathogen exceeded 1 000 cfu/g. Some summary statistics for these four samples are further shown in Table 18, for example the median *L. monocytogenes* count of cfu/g of food was 3 146.

Table 17: Categorised *L. monocytogenes* counts (cfu/g) present in soft or semi-soft cheeses, at the end of shelf-life, in the EU,[(a)] 2010-2011

	***L. monocytogenes* count (cfu/g)**							
	< 10[(b)]	10-39	40-100	> 100-1 000	> 1 000-10 000	> 10 000-100 000	> 100 000	**Total**
Total No of samples	3 448	1	1	0	1	1	0	3 452
Proportion of samples (%)	99.9	0.03	0.03	0	0.03	0.03	0	
Proportion of samples among those with at least 10 cfu/g	-[(c)]	25.0	25.0	0	25.0	25.0	0	

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.
(b): This category includes the samples with results below the limit of detection of the enumeration method. It also includes samples not contaminated with *L. monocytogenes.*
(c): not applicable.

Table 18: Summary statistics of *L. monocytogenes* counts (cfu/g) present in soft or semi-soft cheeses, at the end of shelf-life, for samples with a *L. monocytogenes* count of at least 10 cfu/g, in the EU,[(a)] 2010-2011

Summary statistic	
Total No of samples	4
Geometric mean[(b)]	825
Median[(c)]	3 146
Mode[(d)]	-[(e)]

(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.
(b): Geometric mean is a type of mean or average which is defined as the n^{th} root (where n is number) of the product of the numbers.
(c): Median is the numerical value separating the higher half of the data. For an odd number of observations, the median is the central point of the data. For an even number of observations, there is no single middle value; the median is defined as the mean of the two middle values.
(d): The mode is defined as the value that appears most often in a set of data.
(e) The mode does not exist, as there is no unique count value that appears with a higher frequency than the rest of the count values.

A cross-classification of test results for detection and enumeration tests for soft or semi-soft cheese samples can be found in Appendix G.

6. Discussion

This baseline survey was conducted by 26 MSs and Norway and investigated for the first time foodstuffs at retail. The survey targeted *L. monocytogenes* in RTE food products previously shown to be at risk of contamination at levels considered to be a public health risk, and was not designed to examine the general or overall exposure of EU consumers to *L. monocytogenes* in food. Indeed, the selected categories of RTE foods included in the baseline survey were among RTE foods that have been associated cases of human listeriosis. The risk of exposure to *L. monocytogenes* through consumption of RTE foods is related to the prevalence of *L. monocytogenes* in the food category and the ability of the food to support the growth of the pathogen under the foreseen conditions of storage. Heat treatment at or above 75 °C (or an equivalent time temperature combination, e.g. 70 °C for 2 min.) inactivates *L. monocytogenes*. Processed RTE foods (with or without heat treatment step involved) prone to contamination during processing or further handling and with a prolonged storage time under refrigeration can be high-risk foods as regards *L. monocytogenes*.

The three targeted RTE food categories 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). The samples were randomly selected from the customer display in the retail outlets, based on their availability among a variety of food items belonging to these three food categories. The majority of the samples were taken in supermarkets and small shops in bigger cities and only few samples were taken in other retail outlets (including specialty deli-shops and street or farmers' markets). The sampled foods were kept under refrigeration in participating laboratories for the remaining shelf-life in order to estimate the prevalence and counts of *L. monocytogenes*–contaminated foods at the end of shelf-life, i.e. at stages representing potential consumer exposure, and also the worst –case scenario following further retail or home storage. In addition, the fish samples were also analysed in the laboratory at the time of arrival.

The survey was designed to estimate the EU prevalence of *L. monocytogenes*–contaminated foods in the three targeted RTE food categories, and investigate the counts of *L. monocytogenes* in the sampled foods. Due to the relatively small number of food items sampled per food category in each MS, the data generated by the survey could not be used for obtaining reliable prevalence estimates at the MS level.

In this survey, 6.9 % of samples provided from some smoked or gravad RTE fishery products were found not to support the growth of *L. monocytogenes* owing to their associated intrinsic characteristics of pH and water activity, according to criteria of Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs.[19] But these products represented some types of smoked and gravad RTE fish on the market in the EU. Their inclusion also reflects the challenge faced at an official control level in selecting food products that might support the growth of *L. monocytogenes*, in advance of laboratory analysis to determine water activity and pH. Therefore, it was considered appropriate to maintain these as a valid part of the overall EU dataset.

6.1. *Listeria monocytogenes*-survey results

Ten percent (1 out of 10 samples) smoked or gravad fish samples at the EU level were contaminated with *L. monocytogenes*, at sampling and at the end of shelf-life. For the meat product samples this was 2 % (1 out of 50 samples), whereas for soft and semi-soft cheeses this was 0.5 % (about 1 out of 200 samples), at the end of shelf-life for both.

[19] Products "with pH ≤ 4.4 or $a_w \leq 0.92$, products with pH ≤ 5.0 and $a_w \leq 0.94$ and products with a shelf-life of less than five days" are automatically considered to belong to the category of RTE foods that are unable to support the growth of *L. monocytogenes*.

Overall the presence of low levels of *L. monocytogenes* in foods is not an infrequent event. This is because *L. monocytogenes* is ubiquitous in nature (in soil, vegetation, sewage, surface waters and food-animals). The presence of *L. monocytogenes* in the farm environment, or in the wild fish catch or aquaculture growing areas may represent a primary source for the introduction of the pathogen into the human food supply chain. Contamination often originates in post-processing environments, but contaminated raw foods (raw fish, raw meat, or raw milk) may represent a vehicle for introducing *L. monocytogenes* into food processing plants (Santorum et al., 2012; Di Ciccio et al., 2012). The pathogen has been repeatedly isolated from the environment of food processing plants (Kornacki and Gurtler, 2007), where it can be established in the form of biofilms and, therefore, persist for prolonged periods of time.

The proportion of smoked or gravad fish samples with a *L. monocytogenes* count exceeding the level of 100 cfu/g was, at EU level, 1.7 % (about 1 out of 60 samples) and 1.0 % (about 1 out of 100 samples), respectively, at the end of shelf-life and at sampling. For meat products, this proportion was 0.43 % (15 samples, or about 1 out of 200 samples) while for the cheese it was only 0.06 % (2 samples, or about 1 out of 2 000 samples). These results are mainly consistent with the data reported by MSs in the EU annual monitoring report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the EU. Investigations reported in the EU Summary Reports focus on testing RTE foods for compliance with the food safety criteria for *L. monocytogenes* as laid down by Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. In 2010 and 2011, among single samples collected at retail, the highest levels of non-compliance with the criterion[20] '≤ 100 cfu/g' of Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs were observed in RTE fishery products (respectively 1 % and 0.6 %) and RTE meat products other than fermented sausage (respectively 0.4 % and 0.2 %). Non-compliance was also detected in soft and semi-soft cheeses and was 0.2 % in 2010 and < 0.1 % in 2011 (EFSA and ECDC, 2012, 2013).

The observed EU prevalence of *L. monocytogenes* in smoked and gravad fish as well as in meat products is consistent with the investigations reported by MSs in the EU Summary Reports and with the literature findings. In 2010, MSs reported a prevalence of *L. monocytogenes* contaminated RTE fish and RTE fishery products at retail ranging from 1.5 % to 9.7 %, based on investigations of single samples. For single meat product samples at retail, the prevalence ranged from 0 % to 11.4 % (EFSA and ECDC, 2012). In a survey on the prevalence of *L. monocytogenes* in RTE foods at retail in Sweden in 2010, the pathogen was detected in 12 % of 558 smoked and gravad fish samples at the end of shelf-life (Lambertz et al., 2012). Beaufort et al. (2007) followed-up nine representative cold smoked salmon producing sites in France from 2001 to 2004, and found a mean prevalence of 10.3 % at the end of shelf-life in their products. Corcoran et al. (2006) have indicated the potential for persistence of *L. monocytogenes* in smoked salmon production environments. In contrast to the low differences found in this survey in contamination levels between analysis at sampling as compared to at the end of shelf-life, Beaufort et al. (2007) found low contamination levels at the beginning of shelf-life (only 8 % exceeded 1 cfu/g, with a maximum of 7 cfu/g), and much higher at the end of shelf-life (17 % of the samples exceeded 100 cfu/g, the highest contamination level being 2 800 cfu/g). In a study by Uyttendaele et al. (2009) encompassing 639 samples of a variety of cooked meat products analysed in the period 2005-2007 in Belgium, *L. monocytogenes* was detected in seven samples (1.1 %). None of the samples showed levels above 100 cfu/g. A reduction in prevalence of *L. monocytogenes* was noted compared to a prior survey of cooked meat products (n = 3 405) also in Belgium dating from 1999 (Uyttendaele et al., 1999) in which a 4.9 % prevalence of the pathogen was established. A survey on pâté in Spain found 5.4 % of samples (n = 182) contaminated with *L. monocytogenes* (Dominguez et al., 2001) whereas another study in Spain showed a 7.3 % prevalence (5/68) in sliced cooked meat products (Perez-Rodriguez et al., 2010). In Sweden in a

[20] The implementation of the hygiene package EU-wide microbiological criterion had come into effect in 2006 requiring food business operators producing ready-to-eat foods capable of supporting the growth of *Listeria* to verify the effectiveness of their food safety management systems using a microbiological criterion based around absence of *Listeria* at the end of production, or less than 100 cfu/g at the end of shelf-life.

national survey in 2010 *L. monocytogenes* was detected in 1.2 % of 507 meat-product samples with none of these samples exceeding 100 cfu/g (Lambertz et al., 2012). In Ireland the prevalence of *L. monocytogenes* on slicing machines used for cooked RTE meat products at retail level was found to be 0.23 % (FSAI, 2011).

While the very low number of cheese samples exceeding 100 cfu/g is in concordance with other reports, the observed EU prevalence of *L. monocytogenes*–contaminated soft or semi-soft cheeses was unexpectedly low. The observed prevalence is even more remarkable as the baseline survey samples were analysed at the end of shelf-life, whereas other studies have tested the cheeses at the time of sampling. The observed prevalence of *L. monocytogenes*–contaminated single samples of soft or semi-soft cheeses in the investigations at retail reported by MSs in 2010 in the EU Summary Report ranged from 0 % to 0.7 % (EFSA and ECDC, 2012). Some other reports from MSs found 3 to 5 % of cheese samples positive for *L. monocytogenes* (Rudolf and Scherer, 2001; Wagner et al., 2007). A small survey (n = 137) was recently conducted in Greece targeting soft and semi soft cheeses in retail outlets. The survey was designed according to the Report of the Task Force on Zoonoses Data Collection on the proposed technical specifications for a survey on *L. monocytogenes* (EFSA, 2009a), with the exception that cheeses were examined at the time of sampling and also fresh cheeses were included in the analysis. None of the samples tested positive for *L. monocytogenes* (either qualitatively or quantitatively) (0 %; 95 % CI = 0–2.2 %) (Angelidis et al., 2012). However, a very low prevalence of *L. monocytogenes* in semi-soft cheese at retail was also noted in a national survey in Sweden in 2010 in which *L. monocytogenes* was detected in 0.4 % of 525 cheese samples (Lambertz et al., 2012).

The baseline survey findings show that for soft or semi-soft cheeses it is possible to produce the foods with a rare proportion having counts exceeding the level of 100 cfu/g. It demonstrates that properly designed and implemented food safety management systems by dairy industry across the EU can produce safe compliant food in these categories. Food business operators in these sectors should be aware of the benefits of diligent application of appropriate protocols to manage this particular risk.

In the case of smoked or gravad fish, and to a lesser extent of meat products, the baseline survey findings suggest that the propensity for *L. monocytogenes* to be present might not be adequately addressed through the food safety management systems in production. Consequently, food business operators appear to have more to do in ensuring that their food safety management systems are in fact capable of minimising pre-processing contamination, maximising process listericidal effectiveness, preventing post-processing contamination, and maximising the residual listeriostatic effect of food as placed on the market. The effectiveness of food safety management systems should be evaluated by verifying compliance with *L. monocytogenes* microbiological criteria.

Although the proportions of RTE foods found in this survey with a *L. monocytogenes* count exceeding the level of 100 cfu/g were low, very low or rare, it should be mentioned that investigations on outbreaks of listeriosis report all three investigated RTE food categories as the source of infection. Typically, in the EU annual monitoring report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the EU, no or very few food-borne outbreaks caused by *L. monocytogenes* are recorded by MSs, and cheese was the main implicated vehicle reported by MSs in 2009 and 2011. More precisely, in 2009 three *L. monocytogenes* food-borne outbreaks were reported by MSs and one of these was a multinational outbreak caused by cheese and covering cases from three MSs (EFSA and ECDC, 2011). The incriminated food vehicles in the reported three strong evidence *L. monocytogenes* food-borne outbreaks in 2010 were fish and fish products, other or mixed meat, and one outbreak was from an unspecified food source (EFSA and ECDC, 2012). *L. monocytogenes* outbreaks have the highest case fatality rates of all the agents associated with food-borne outbreaks reported annually by MSs, emphasising the importance of the strict control of *L. monocytogenes* in foodstuffs. Finally, in 2011, four outbreaks with strong evidence for *L. monocytogenes* were reported implicating domestically produced cheese, bakery products, mixed food and pig meat and products thereof (EFSA and ECDC, 2013).

6.2. Comparisons of results of survey tests

A low proportion of fish and meat product samples, as well as one cheese sample, were positive by the enumeration test, but detection-test negative. Such results might look conflicting, since the detection method should be more sensitive than the enumeration method. These findings might be due to the "competition" between *L. monocytogenes* (present in low numbers) and other *Listeria* spp. (typically *L. innocua*, present at equal or higher levels) present in these foods. It has been shown than *L. monocytogenes* competes rather poorly in the presence of *L. innocua* during the enrichment steps in *L. monocytogenes*-enrichment broths (Gnanou Besse et al., 2005, 2010; Yokoyama et al., 2005; Oravcova et al., 2008; Zitz et al., 2011). Note that in the present survey, only the presence and counts of *L. monocytogenes* were sought, but not those of other *Listeria* spp. Second, the food contamination heterogeneity could also explain these findings. It is known that the homogenization step in the laboratory using a stomacher, as prescribed in this survey, minimizes the contamination "heterogeneity" but does not eliminate it, in particular if the contamination is very low. Finally, experimental/technical error can not be excluded either and there may also be other, currently unknown, factors/causes for this phenomenon.

Observations were also made on the concordance and discordance of the EU fish survey prevalence and enumeration results at the end of shelf-life compared with at the time of sample collection. These can be explained by the contamination heterogeneity within a batch and the fact that the analysis at the time of sampling and the analysis at the end of shelf-life necessitated two different packages from the same batch being analysed. Still, these differences will be investigated further in the Report Part B taking account of the important variability of the remaining shelf-life of the surveyed fish samples and possible other factors that may contribute to the observed differences. The Part B report, which will also report on the comparisons of results among fish subcategories, will be published at a later stage.

6.3. Relevance of the findings to human health

Among the recognized species of the genus *Listeria*, *L. monocytogenes* is essentially the only pathogenic species for humans. Human cases of listeriosis are usually sporadic, but outbreaks of various magnitudes also occur. The disease usually manifests itself as febrile gastroenteritis in otherwise healthy human hosts, but also as an invasive disease in high-risk individuals. Although the incidence of invasive listeriosis in developed countries is rather low, the disease is severe, with a high (20-30 %) mortality rate. The risk of invasive listeriosis is higher among certain population groups such as the elderly, pregnant women, neonates and patients under iatrogenic immune-suppression, as well as patients with underlying immune-suppressive conditions (Painter and Slutsker, 2007). According to the EU summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011 (EFSA and ECDC, 2013), 26 EU MSs reported 1 476 confirmed cases of human listeriosis, with an incidence of 0.32 cases per 100 000 individuals. Sixteen MSs provided information on hospitalisation for listeriosis for all or the majority of their cases and on average 93.6 % of the cases were hospitalised, in 2011. In ten MSs this proportion was 100 %. This is the highest hospitalisation of all zoonoses under EU surveillance. A total of 134 deaths due to listeriosis were reported by 19 MSs in 2011 resulting in an EU case fatality rate of 12.7 %.

Listeriosis acquired from food is mostly due to the consumption of RTE foods which support the growth of *L. monocytogenes* and develop a high concentration of *L. monocytogenes* along the food chain. The ability of *L. monocytogenes* to proliferate under refrigeration temperatures is probably the most salient feature of the pathogen, given that refrigeration is the most commonly used method of food preservation in developed countries. Since RTE foods do not require any bactericidal treatment on behalf of the consumer prior to consumption, contamination of RTE foods that can support the growth of *L. monocytogenes* can pose public health risks.

Recent risk assessment concluded that most listeriosis cases are due to foods having a *L. monocytogenes* count markedly above the level of 100 cfu/g. The impact on public health would depend on whether levels greatly exceeding 100 cfu/g are reached (EFSA 2007b). To protect public

health a count exceeding the level of 100 cfu/g at the end of the product's shelf-life is considered unsafe in EU legislation and products containing such levels must be withdrawn or recalled from the market.

The results of this baseline survey show that, at the end of shelf-life, a low proportion of smoked and gravad fish samples contained counts exceeding the food safety limit of 100 cfu/g. This is of concern to public health as the risk for human listeriosis increases with increasing numbers of ingested cells. For meat products and cheeses respectively, a very low and rare proportion was observed. However, taking into account the popularity of these meat and cheese products, these results may be still a concern for public health. It is noteworthy that the 'time of sampling' concept in this survey was a random and arbitrary point in the shelf-life of the RTE food products and can be regarded as a typical time at which these products are available for retail purchase. However, products purchased at this point followed by home storage might be expected to produce higher counts at end of shelf-life as a result of temperature abuse. In addition and of cause for concern, work by the UK Advisory Committee on the Microbiological Safety of Food (ACMSF) has shown a disregard for dates of minimum durability (i.e. use-by-dates) of RTE products in some sectors of the population at risk for listeriosis (ACMSF, 2009). On the other hand, it seems that, in general, the surveyed food samples were stored at the laboratory under satisfactory temperature conditions; therefore, in this sense providing scenario of the presence of *L. monocytogenes* in the surveyed foods at the end of shelf-life that was not worst case.

Good manufacturing practices, appropriate cleaning, sanitation and hygiene programs and effective temperature control throughout the food production, distribution and storage chain are required for prevention of contamination or inhibition of growth of the pathogen to levels exceeding 100 cfu/g in foods that may pose a *L. monocytogenes* risk. An effective food safety management system implemented by trained staff is important to control the prevalence and numbers of *L. monocytogenes* in these at risk food products. Consumers can protect themselves by following storage instructions and respecting use-by-dates as *L. monocytogenes* can grow at refrigeration temperatures. The consumers, particularly the vulnerable groups such as pregnant women and the elderly and chronically ill, who are more susceptible to invasive listeriosis, are also advised to follow the guidelines given by the national authorities regarding the consumption of foodstuffs related to higher risk of *L. monocytogenes* contamination.

The data provided by this survey, gathered in all EU countries using a similar and representative nationwide sampling plan, will be useful in assessing the exposure of EU consumers to *L. monocytogenes* via the three specific RTE food categories.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

- This baseline survey investigated RTE foods at retail and it was designed to yield prevalence estimates only at the EU level. The survey was not designed to yield MS-specific *L. monocytogenes* prevalence estimates in these RTE foods, and the results do not necessarily represent MSs-specific situations.
- This was the first time that these particular RTE products/food commodities potentially supporting *L. monocytogenes* growth were investigated using a similar and representative nationwide sampling plan. The RTE products/food commodities were found to have quite a variation in intrinsic characteristics (pH, water activity) and remaining shelf-life.
- The EU prevalence of *L. monocytogenes*-contaminated fish samples at the time of sampling was 10.4 %, while at the end of shelf-life it was 10.3 %. Overall, it can be noted that the prevalence of *L. monocytogenes* in smoked and gravad fish found in this survey corresponds to what is reported in the literature for this food category. The EU level proportion of samples exceeding the level of 100 cfu/g at sampling was 1.0 %, while for fish at end of shelf-life it was 1.7 %. This is of concern to public health as the risk of human listeriosis increases with increasing numbers of ingested cells.
- In meat products, the EU prevalence of *L. monocytogenes*-contaminated samples at the end of shelf-life was 2.07 %, while the EU level proportion of samples exceeding the level of 100 cfu/g was 0.43 %. While these results are not unexpected, they still give cause for concern, especially considering the popularity of these types of at-risk food category in the consumption pattern of EU consumers.
- The EU prevalence of *L. monocytogenes*-contaminated soft and semi-soft cheese samples at the end of shelf-life was 0.47 % and the EU level proportion of samples exceeding the level of 100 cfu/g was 0.06 %. While the rare proportion of the cheese samples exceeding 100 cfu/g is in concordance with other reports, the percentage of cheese qualitatively positive for *L. monocytogenes* was unexpectedly low. These data show that, even for a food category with the potential to be contaminated by this organism, it is possible to achieve meaningful risk management and produce a safe end-product. However, even a rare proportion of samples exceeding the level of 100 cfu/g may raise concern for public health.
- The data provided by this survey, gathered in all EU countries using a similar and representative nationwide sampling plan, will be useful in assessing the exposure of EU consumers to *L. monocytogenes* via the three specific RTE food categories thought to pose a particular risk for *L. monocytogenes*.

RECOMMENDATIONS

- Good manufacturing practices, appropriate cleaning, sanitation and hygiene programs and effective temperature control throughout the food production, distribution and storage chain are required for prevention of contamination or inhibition of growth of *L. monocytogenes* to levels exceeding 100 cfu/g in foods that may pose a *L. monocytogenes* risk.
- Food business operators producing RTE smoked and gravad fish should be aware of the particular challenges, which appear not to have been overcome, in ensuring acceptable *L. monocytogenes* risk management.
- The surveyed foods were RTE and therefore, are intended to be consumed without any further heat treatment. The findings indicate the ongoing presence of *L. monocytogenes* in such foods. All food business operators and consumers should keep the temperatures of their refrigerators low, in order to limit potential growth of *L. monocytogenes* if this is present in RTE products.
- It would be beneficial to remind consumers about the importance of following the manufacturers' storage instructions respecting use-by-dates, and of following the guidelines given by the national authorities on consumption of the foodstuffs in question.
- If the estimation of *L. monocytogenes* prevalence at the MS level and the assessment of potential variability of this prevalence among MSs was of interest, more detailed surveys of RTE foods with larger sample sizes would be required. Collection of detailed information on the production date, on pH, water activity, additives (including their concentrations) and other antimicrobial hurdles is very important for the assessment of the growth potential of *L. monocytogenes* in the surveyed foods and this information should be gathered where possible in future studies.

REFERENCES

ACMSF (Advisory Committee on Microbiological Safety of Food), 2009. Report on the increased incidence of Listeriosis in the UK. Available online: http://www.food.gov.uk/multimedia/pdfs/committee/acmsflisteria.pdf

Angelidis AS, Georgiadou SS, Zafiropoulou V, Velonakis EN, Papageorgiou DK and Vatopoulos A, 2012. A survey of soft cheeses in Greek retail outlets highlights a low prevalence *of Listeria* spp. Dairy Science and Technology, 92,189-201.

Beaufort A, Rudelle S, Gnanou-Besse N, Toquin MT, Kerouanton A, Bergis H, Salvat G and Cornu M, 2007. Prevalence and growth of *Listeria monocytogenes* in naturally contaminated cold-smoked salmon. Letters in Applied Microbiology,44, 406-411.

CAC (Codex Alimentarius Commission), 2008. Codex general standard for cheese. CODEX STAN 283-1978. Formerly CODEX STAN A-6-1973. Adopted in 1973. Revision 1999, Amendments 2006, 2008. English version, 7 pp. Available online: http://www.codexalimentarius.org/input/download/standards/175/CXS_283e.pdf

Corcoran D, Clancy D, O'Mahony M, Grant K, Hyland E, Shanaghy N, Whyte P, McLauchlin J, Moloney A and Fanning S, 2006. Comparison of *Listeria monocytogenes* strain types in Irish smoked salmon and other foods. International Journal of Hygiene and Environmental Health, 209, 527–534.

Di Ciccio P, Meloni D, Festino AR, Conter M, Zanardi E, Ghidini S, Vergara A, Mazzette R and Ianieri A, 2012. Longitudinal study on the sources of *Listeria monocytogenes* contamination in cold-smoked salmon and its processing environment in Italy. International Journal of Food Microbiology, 158, 79-84.

Dominguez C, Gomez I and Zumalacarregui J, 2001. Prevalence and contamination levels of *Listeria monocytogenes* in smoked fish and pate sold in Spain. Journal of Food Protection, 64, 2075-2077.

Dupont C and Augustin JC, 2009. Influence of stress on single-cell lag time and growth probability for *Listeria monocytogenes* in half Fraser broth. Applied and Environmental Microbiology, 75, 3069-3076.

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, 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

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2011. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2009. EFSA Journal 9(3):2090. [378 pp.] doi:10.2903/j.efsa.2011.2090.

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2012. The European Union Summary Report on Trends and Sources of Zoonoses,

Zoonotic Agents and Food-borne Outbreaks in 2010. EFSA Journal 10(3):2597. [442 pp.] doi:10.2903/j.efsa.2012.2597.

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, 11(4):3129, 250 pp. doi:10.2903/j.efsa.2013.3129.

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 online: http://www.fsai.ie/establishingbaselinedataonthepresenceoflisteriamonocytogenesoncookedmeatslicersinretailandcateringpremises.html

Gnanou Besse N, Audinet N, Kérouanton A, Colin P and Kalmokoff M, 2005. Evolution of *Listeria* populations in food samples undergoing enrichment culturing. International Journal of Food Microbiology, 104, 123-134.

Gnanou Besse N, Barre L, Buhariwalla C, Vignaud ML, Khamissi E, Decourseulles E, Nirsimloo M, Chelly M and Kalmokoff M, 2010. The overgrowth of *Listeria monocytogenes* by other *Listeria* spp. in food samples undergoing enrichment cultivation has a nutritional basis. International Journal of Food Microbiology, 136, 345-351.

Kornacki JL and Gurtler JB, 2007. Incidence and control of *Listeria* in food processing facilities. In: *Listeria*, listeriosis and food safety, third edition. Eds Ryser ET and Marth EH. CRC Press, Boca Raton, 681-766.

Lambertz ST, Nilsson C, Brådenmark A, Sylvén S, Johansson A, Jansson LM and Lindblad M, 2012. Prevalence and level of *Listeria monocytogenes* in ready-to-eat foods in Sweden 2010. International Journal of Food Microbiology, 160, 24-31.

Loncarevic S, Økland M, Sehic E, Norli HS and Johansson T, 2008. Validation of NMKL method No. 136 - *Listeria monocytogenes*, detection and enumeration in foods and feed. International Journal of Food Microbiology, 124, 154-163.

Oravcova K, Trncikova T, Kuchta T and Kaclikova E, 2008. Limitation in the detection of *Listeria monocytogenes* in food in the presence of competing *Listeria innocua*. Journal of Applied Microbiology, 104, 429-437.

Painter J and Slutsker L, 2007. Listeriosis in humans. In: *Listeria*, listeriosis and food safety, third edition. Eds Ryser ET and Marth EH. CRC Press, Boca Raton, 85-109.

Perez-Rodriguez F, Castro R, Posada-Izquierdo GD, Valero A, Carrasco E, Garcia-Gimeno RM and Zurera G, 2010. Evaluation of hygiene practices and microbiological quality of cooked meat products during slicing and handling at retail. Meat Science, 86, 479-485.

Rakhmawati T W, Nysen R, Aerts M, 2013. Statistical analysis of the *Listeria monocytogenes* EU-wide baseline survey in certain ready-to-eat foods Part A: *Listeria monocytogenes* prevalence estimates. EFSA supporting publication 2013:EN-441, 114 pp.

Rijpens N and Herman L, 2004. Comparison of selective and nonselective primary enrichments for the detection of *Listeria monocytogenes* in cheese. International Journal of Food Microbiology, 94, 15-22.

Rudolf M and Scherer S, 2001. High incidence of *Listeria monocytogenes* in European red smear cheese. International Journal of Food Microbiology, 63, 91-98

Santorum P, Garcia R, Lopez V and Martinez-Suarez JV, 2012. Review. Dairy farm management and production practices associated with the presence of *Listeria monocytogenes* in raw milk and beef. Spanish Journal of Agricultural Research, 10, 360-371.

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.

Uyttendaele M, Busschaert P, Valero A, Geeraerd AH, Vermeulen A, Jacxsens L, Goh KK, De Loy A, Van Impe JF and Devlieghere F, 2009. Prevalence and challenge tests of *Listeria monocytogenes* in Belgian produced and retailed mayonnaise-based deli-salads, cooked meat products and smoked fish between 2005 and 2007. International Journal of Food Microbiology, 133, 94-104.

Wagner M, Auer B, Trittremmel C, Hein I and Schoder D, 2007. Survey on the *Listeria* contamination of ready-to-eat food products and household environments in Vienna, Austria. Zoonoses and Public Health, 54, 16-22.

Yokoyama E, Shibusawa Y, Maruyama S, Katsube Y and Mikami T, 2005. Influence of bacteriocin-like substance, generation times, and genetic profiles of *Listeria innocua* on the isolation of *Listeria monocytogenes*. Comparative Immunology, Microbiology & Infectious Diseases, 28, 177-186.

Zitz U, Zunabovic M, Domig KJ, Wilrich PT and Kneifel W, 2011. Reduced detectability of *Listeria monocytogenes* in the presence of *Listeria innocua*. Journal of Food Protection, 74, 1282-1287.

APPENDICES

Appendix A. Data dictionary for 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. Performance of the analytical methods

Appendix B.1. Detection method

Detection of *L. monocytogenes* was performed according to EN ISO 11290-1:1996 amended in 2004 (EN ISO 11290-1:1996/A1:2004).

Briefly, the method consists of a double enrichment in Half Fraser and Fraser selective broths. The initial incubation in Half Fraser broth is carried out for 24 hours at 30 °C. The second step of the enrichment is carried out in Fraser broth for 48 hours at a temperature of 37 °C. Half Fraser broth contains half the concentration of nalidixic acid and acriflavin of that found in Fraser broth. Cultures obtained in Half Fraser and Fraser broths are plated out on two selective solid media: Agar *Listeria* according to Ottaviani and Agosti and an additional selective medium of own choice. After appropriate incubation, the colonies of presumptive *L. monocytogenes* or *Listeria* species, are sub-cultured and confirmed by means of appropriate morphological and biochemical tests described in the Standard.

The theoretical limit of sensitivity of the EN ISO 11290-1 method for the detection of *L. monocytogenes* in food is one cell in 25g or ml samples (i.e. 0.04/g). From an experimental point of view, the limit of detection of the method is indeed very low, close to the theoretical value.

The relative level of detection (LOD_{50}) is the smallest number of culturable microorganisms that can be detected in the sample in 50 % of occasions by the alternative and reference methods. In 2012, 22 validation studies of rapid commercial methods performed in comparison to the Standard method were available from AFNOR Certification for the detection of *L. monocytogenes* in food and environmental samples (www.afnor-validation.org). According to these studies, the standard method shows a LOD generally below 1, comprised between of 0.4 and 1.7 cfu/25 g for meat, between 0.3 and 1.3 for seafood products and between 0.3 and 1.2 cfu/25 g for dairy products.

The validation study of the revised Nordic Committee on Food Analysis (NMKL) method N°136 (Loncarevic et al., 2008), very similar to the EN ISO 11290-1 Standard, allowed to better define the performance characteristics of the method. The sensitivity values of the detection method were 98.6 %, 97.2 % and 98.6 %, respectively, for brie cheese made from pasteurised milk, hot smoked salmon and cooked vacuum-packed ham, and the specificity values were respectively 94.4 %, 100 % and 100 % for the same products.

While the method is very effective, it is believed that the double enrichment may allow overgrowth of *L. monocytogenes* by *L. innocua* in samples where both species are present. Indeed, each of the species within the genus *Listeria* can be isolated from food. From a practical perspective, the overgrowth by a non-pathogenic species of *Listeria* may mask the presence of low numbers of *L. monocytogenes* in the original food sample, and result in false-negative results (Gnanou Besse et al., 2005, 2010; Oravcova et al., 2008; Zitz et al., 2011). In the present survey, no information is available concerning the presence and prevalence of other *Listeria* spp. in the samples.

Although conflicting results are reported on this subject, the Standard selective enrichment procedure may also lead to lower recovery of the injured bacteria (Rijpens and Herman, 2004; Dupont and Augustin, 2009).

Appendix B.2. Enumeration method

The enumeration of *L. monocytogenes* was performed according to EN ISO 11290-2:1998 and its modification EN ISO 11290-2:1998/A1:2004.

Briefly, the test portion is decimally diluted in an appropriate diluent (buffered peptone water or half Fraser broth base without selective agents) and subsequently homogenised. A specified volume of this initial suspension and/or of subsequent decimal dilutions is surface-plated on Agar *Listeria* according to Ottaviani and Agosti. After appropriate incubation, the colonies of presumptive *L. monocytogenes* are counted, sub-cultured and confirmed by means of appropriate morphological and biochemical tests described in the Standard. Calculation of the *L. monocytogenes* contamination level is carried out according to the number of confirmed colonies.

For the dilution of cheese, a sodium citrate solution, as described in EN ISO 6887-5[21] may be used as diluent.

According to the expected low contamination levels, it was advised to plate 1 ml of the initial suspension in duplicate on three 90-mm plates (or one plate of 140 mm diameter), as indicated in the Standard, in order to increase theoretical limit of sensitivity to10 cfu/g.

Moreover, for fish samples tested on arrival at the laboratory, the determination of the pH of the sample should be performed according to EN ISO 2917:1999, while the determination of the water activity of the sample should be performed according to EN ISO 21807:2004.

The theoretical limit of sensitivity of the EN ISO 11290-2 method for the enumeration of *L. monocytogenes* in food is 10 cfu/g when spreading 1 ml of the decimally diluted sample on three 90-mm plates (or one plate of 140 mm diameter).

According to ISO 7218:2007 Standard:

- the limit of detection of the method is 10 cfu/g (when spreading 1 ml of the initial food suspension).

- the theoretical limit of quantification is then 40 cfu/g (four times the limit of detection). Below this value, the microorganism cannot be reliably quantified, though its presence may be reported.

- under 100 cfu/g (which correspond to 10 colonies when spreading 1 ml of the initial food suspension) the result has to be expressed as an estimated result or its measurement uncertainty has to be specified.

Moreover, a contamination level of about 100 cfu/g (when spreading in duplicate 1 ml of the initial food suspension) is also associated with a quite elevated 95% confidence interval (up to 60 to 150 cfu/g according to ISO 7218:1996,[22] Annex A: Table for confidence interval for low number estimation).

The validation study of the revised NMKL (Nordic Committee on Food Analysis) method No 136 (Loncarevic et al., 2008), very similar to EN ISO 11290-2 Standard, allowed to determine the precision of the method in terms of repeatability (r) and reproducibility (R) using different food sample types (brie cheese from pasteurised milk, hot smoked salmon and cooked vacuum-packed ham). For a contamination level close to 100/g (2.2 $\log_{10}$ cfu/g) the overall repeatability of the method for these products was r = 0.44, 0.91 and 0.66, respectively, and the overall reproducibility was R = 0.48, 1.08 and 0.54, respectively. In the presence of *L. innocua*, these values reached, respectively, r = 0.76, 0.52, 0.70 and R = 0.87, 0.68, 0.87. This means that for a sample having a true contamination

[21] EN ISO 6887-5. Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 5: Specific rules for the preparation of milk and milk products.
[22] ISO 7218:1996. Microbiology of food and animal feeding stuffs – General rules for microbiological examinations.

level of 100 cfu/g, if $R = 0.87$ a laboratory may find a result down to 13 cfu/g, and another one a result up to 741 cfu/g.

Such variability is a common feature to all the enumeration methods used in food microbiology, and based on counts of colonies on Petri dishes. However, in the context of low *L. monocytogenes* contamination levels in food (≤ 100/g), most results of the present survey are associated with high variability, and should be handled carefully, though they provide useful information on the overall distribution of contamination levels.

Appendix C. Exclusion criteria for coordinated monitoring programme for *Listeria monocytogenes* in certain ready-to-eat food categories at retail Version 4.1 dated 15 December 2010

The purpose is to integrate as much as possible the exclusion criteria in the reporting forms in order to alert the reporting officer immediately when a draft report is not in line with all exclusion criteria.

Criterion No	Criterion	Rationale for the criterion
General Criterion	is null (empty)	This criterion excludes all records with one or more mandatory fields 'NULL (EMPTY)'.
1	003 Code of retail outlet, 007 Reference of the sample: no special characters allowed (!"£$%^&*())_+}]~#@':;?/>.<,|\), space and tab	This criterion excludes all records including special characters, space or tab.
2	005 Date of sampling: < 15 December 2009	This criterion excludes all records containing a date of sampling before 15 December 2009.
3	005 Date of sampling: > 15 January 2012	This criterion excludes all records containing a date of sampling after 15 January 2012.
4	027 Use by Date: < 15 December 2009	This criterion excludes all records containing a date of use by date before 15 December 2009.
5	018, 033 'Date of testing' not before 005 'Date of sampling'	This criterion excludes records with a date of testing before the date of sampling.
6	033 'Date of testing at the end of shelf-life' not before 018 'Date of testing for fish product on the arrival at the laboratory'	This criterion excludes records with a date of the end of shelf-life before the date of testing on the arrival at the laboratory.
7	031 'Storage temperature at retail': < 0 or > 30 °C	This criterion excludes all records with a storage temperature at retail below 0 °C or above 30 °C (included).
8	Difference date between: '033 Date of testing at the end of the shelf-life' and '027 Use by day': ≥ 4	This criterion excludes all records containing a difference between the use by date and the date of testing at the end of the shelf-life (or vice versa) above 4 days. However, four day difference is only allowed in exceptional cases, like long weekends.

Table continued overleaf.

Appendix C (continued). Exclusion criteria for coordinated monitoring programme for *Listeria monocytogenes* in certain ready-to-eat food categories at retail

Criterion No	Criterion	Rationale for the criterion
9	019, 034 *L. monocytogenes* quantification result at the end of the shelf-life: is not an integer (contains spaces, comma, dot or any other alphanumerical character).	This criterion excludes all records with a value for quantification result that is not an integer number.
10	019, 034 *L. monocytogenes* quantification result at the end of the shelf-life: > 0 and < 10	This criterion considers values from 0 to 9 (included) to be equal to 0
11	021 pH test result at the sampling stage: < 0 and > 14	This criterion excludes all records containing a decimal number integer below 0 or above 14 (included).
12	022 Water activity (a_w) result at the sampling stage: < 0.88 or > 1.00	This criterion excludes all records containing a decimal number integer below 0.88 or above 1.00 (included).
13	For all alphanumeric values no special characters should be allowed (!"£$%^&*()_+}]~#@';:?/) including space, tab and control	
14	031 Storage temperature at retail and 036 Storage temperature at laboratory up to the end of shelf-life (ºC) < 0 ºC or > 30 ºC: Only the values from 0 to 30 (included) ºC should be allowed	
15	028, 029 "Packaging date" should not be before "production date"	This criterion excludes all records with the packaging date before the production date.

Appendix D. Achieved sample sizes as compared to the planned sample sizes for the survey, for the surveyed food categories

Country	Planned sample size		Achieved sample size				
	No per food category	Total No	Smoked or gravad fish at sampling	Smoked or gravad fish at end of shelf-life	Heat treated meat products at end of shelf-life	Soft or semi-soft cheese at end of shelf-life	Total
Austria	60	240	128	128	123	129	508
Belgium	60	240	27	27	27	16	97
Bulgaria	60	240	45	45	39	42	171
Cyprus	30	120	27	27	27	27	108
Czech Republic	60	240	12	12	60	60	144
Denmark	60	240	60	60	60	49	229
Estonia	30	120	30	30	30	30	120
Finland	60	240	63	63	66	65	257
France	400	1 600	391	391	389	415	1 586
Germany	400	1 600	474	474	915	829	2 692
Greece	60	240	59	59	60	58	236
Hungary	60	240	61	61	62	54	238
Italy	400	1 600	389	389	403	398	1 579
Ireland	30	120	31	31	32	35	129
Latvia	30	120	29	29	30	29	117
Lithuania	30	120	30	30	30	30	120
Luxembourg	30	120	22	22	26	27	97
Malta	30	120	36	36	22	19	113
Netherlands	60	240	66	66	56	58	246
Poland	200	800	200	200	200	200	800
Portugal	60	240	-	-	-	-	0
Romania	60	240	60	60	60	60	240
Slovakia	60	240	60	60	59	59	238
Slovenia	30	120	29	29	32	33	123
Spain	200	800	202	202	201	206	811
Sweden	60	240	67	67	75	67	276
United Kingdom	400	1 600	396	396	386	398	1 576
EU	**3 020**	**12 080**	**2 994**	**2 994**	**3 470**	**3 393**	**12 851**
Norway	-	-	59	59	60	59	237
Total	**3 020**	**12 080**	**3 053**	**3 053**	**3 530**	**3 452**	**13 088**

Appendix E. Additional sample descriptions, based on reported parameters

Table 1: Distribution of the country of production for the surveyed food samples, for the *L. monocytogenes* baseline survey, in the EU,[(a)] 2010-2011

Country of production	Fish at sample collection	Fish at the end of shelf-life	Heat treated meat products	Soft or semi-soft cheese
Argentina	-	-	3	-
Austria	28	28	126	88
Belarus	1	1	-	-
Belgium	9	9	66	15
Brazil	-	-	19	-
Bulgaria	39	39	37	30
Canada	2	2	-	-
Croatia	3	3	1	1
Cyprus	14	14	27	4
Czech Republic	22	22	87	60
Denmark	176	176	30	104
Estonia	32	32	38	14
European Union	-	-	1	-
Faroe Islands	1	1	-	-
Finland	44	44	62	19
France	455	455	380	1 389
Germany	168	168	991	509
Greece	61	61	61	106
Greenland	5	5	-	1
Hungary	11	11	63	33
Ireland	29	29	37	17
Israel	-	-	2	-
Italy	75	75	440	480
Latvia	49	49	19	12
Lithuania	116	116	32	16
Luxembourg	1	1	14	1
Malta	-	-	5	-
New Zealand	-	-	-	1
Norway	246	246	59	17
Oman	-	-	1	-
Pakistan	-	-	1	-
Poland	547	547	200	205
Portugal	-	-	-	1
Romania	60	60	45	14
Slovakia	9	9	18	29
Slovenia	6	6	12	3
Spain	200	200	204	47
Sweden	59	59	70	9
Switzerland	1	1	-	29
Thailand	-	-	8	-
Netherlands	55	55	51	32
Turkey	49	49	-	-
Ukraine	1	1	-	-
United Kingdom	471	471	302	166
United States	3	3	18	-
Vietnam	5	5	-	-
Total	**3 053**	**3 053**	**3 530**	**3 452**

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

Note: 'Country of production' is defined in Commission Decision 2010/678/EU as: 'country of production' means the country indicated on the identification mark as provided for in point 6 of Part B

of Section I of Annex II to Regulation (EC) No 853/2004 laying down specific hygiene rules for food of animal origin.

Table 2: Distribution of the variable: 'Transport protocol', indicating whether transport of the samples was carried out in line with technical specifications, for surveyed food samples, for the *L. monocytogenes* baseline survey, in the EU,[(a)] 2010-2011

Transport protocol	Hot or cold smoked or gravad fish	%	Packaged heat-treated meat products	%	Soft or semi-soft cheeses	%
Yes	3 052	> 99.9	3 526	99.9	3 449	99.9
No	1	< 0.1	4	0.1	3	0.1
Total	**3 053**		**3 530**		**3 452**	

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

Table 3: Suitability for human consumption at end the of shelf-life, on the basis of visual and smell evaluation, for surveyed food samples, for the *L. monocytogenes* baseline survey, in the EU,[(a)] 2010-2011

Suitability for human consumption at end of shelf-life	Hot or cold smoked or gravad fish	%	Packaged heat-treated meat products	%	Soft or semi-soft cheeses	%
No	56	2.0	47	1.4	43	1.3
Yes	2 723	98.0	3 219	98.6	3 167	98.7
Unknown[(b)]	274		264		242	
Total	**3 053**		**3 530**		**3 452**	

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

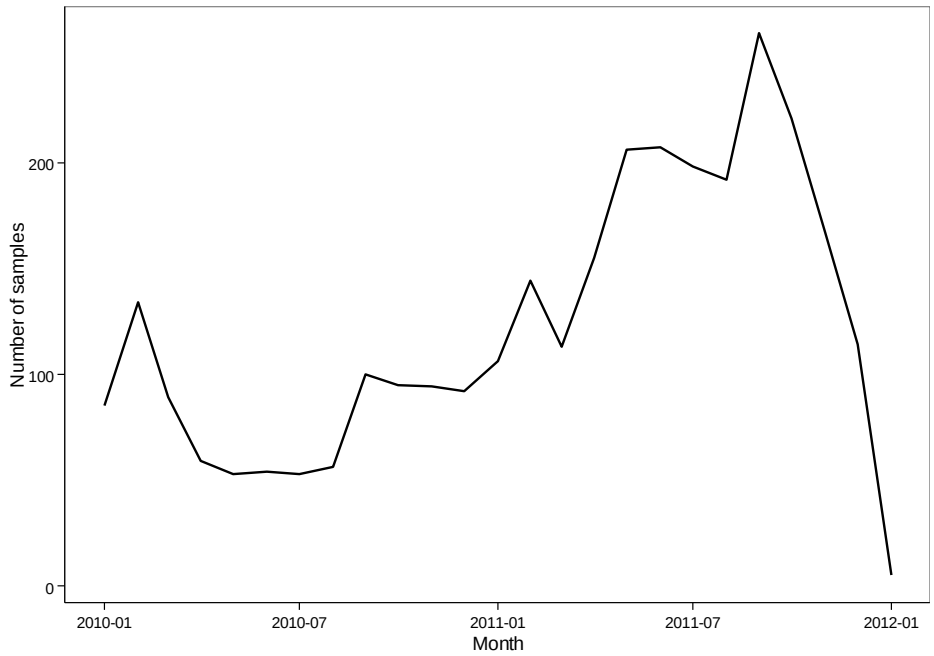
(b): 'Suitability for human consumption at end of shelf-life' was an optional variable; therefore, this information was not reported for all surveyed food samples. Percentages for the categories 'No' and 'Yes' were calculated after excluding the samples for which this information was not reported.

Table 4: Number of samples taken by type of sample by month for the *L. monocytogenes* baseline survey, in the EU,[(a)] 2010-2011

Month	Hot or cold smoked or gravad fish		Packaged heat-treated meat products		Soft or semi-soft cheeses	
	N	%	N	%	N	%
Jan-10	85	2.8	106	3	88	2.5
Feb-10	134	4.4	161	4.6	146	4.2
Mar-10	89	2.9	98	2.8	187	5.4
Apr-10	59	1.9	72	2	69	2
May-10	53	1.7	114	3.2	62	1.8
Jun-10	54	1.8	105	3	70	2
Jul-10	53	1.7	86	2.4	76	2.2
Aug-10	56	1.8	101	2.9	101	2.9
Sep-10	100	3.3	115	3.3	157	4.5
Oct-10	95	3.1	117	3.3	95	2.8
Nov-10	94	3.1	123	3.5	120	3.5
Dec-10	92	3	104	2.9	96	2.8
Jan-11	106	3.5	130	3.7	129	3.7
Feb-11	144	4.7	143	4.1	138	4
Mar-11	113	3.7	121	3.4	131	3.8
Apr-11	155	5.1	189	5.4	147	4.3
May-11	206	6.7	202	5.7	211	6.1
Jun-11	207	6.8	210	5.9	203	5.9
Jul-11	198	6.5	225	6.4	261	7.6
Aug-11	192	6.3	219	6.2	229	6.6
Sep-11	261	8.5	265	7.5	269	7.8
Oct-11	221	7.2	252	7.1	232	6.7
Nov-11	167	5.5	170	4.8	145	4.2
Dec-11	114	3.7	97	2.7	86	2.5
Jan-12	5	0.2	5	0.1	4	0.1
Total	**3 053**		**3 530**		**3 452**	

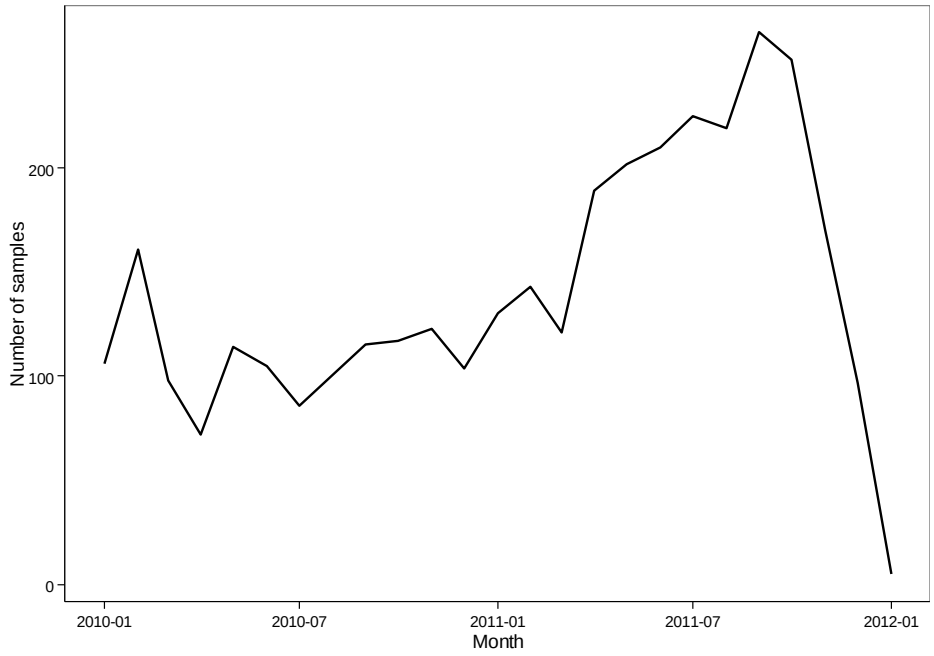
(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.

As can be seen in Table 4, the number of samples taken for the survey was distributed throughout the survey period; however, it peaked between May and November of 2011



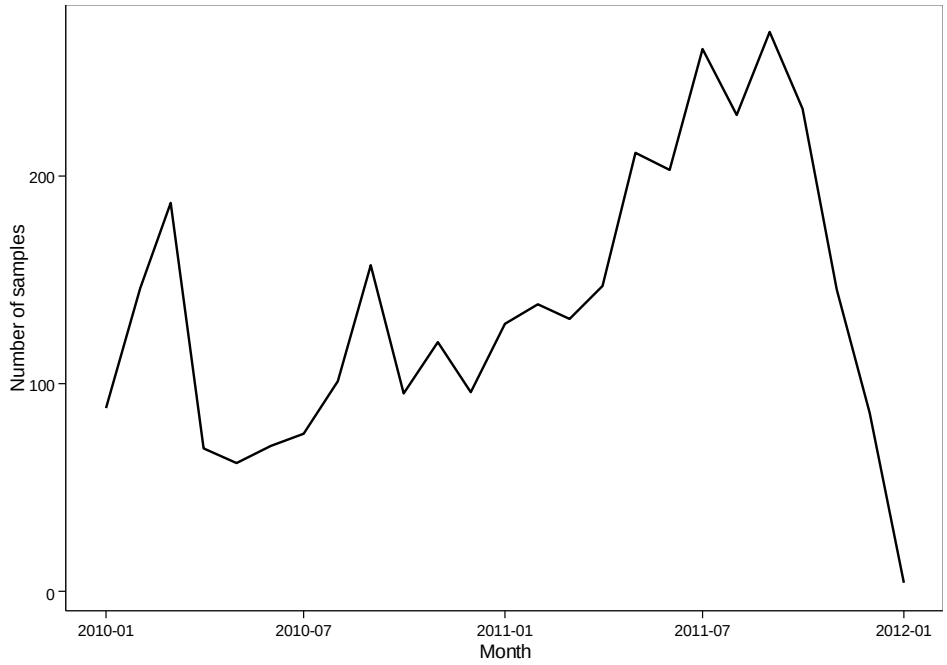
(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.

Figure 3: Number of packaged hot or cold smoked or gravad fish samples taken each month of the survey in the EU,[(a)] 2010-2011



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

Figure 4: Number of packaged heat-treated meat product samples taken each month for the *L. monocytogenes* baseline survey in the EU,[(a)] 2010-2011.



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

Figure 5: Number of soft or semi-soft cheese samples taken each month for the *L. monocytogenes* baseline survey in the EU,[(a)] 2010-2011.

Concerning the subtype of the fish product, over 50 % of the samples were unknown smoked fish, while the second most frequent category was cold smoked fish (Table 5). Concerning the type of retail outlet from which the samples were taken, this was predominantly supermarket or small shop, while most of the fish samples were vacuum packaged, however a substantial proportion was also found to be normal or modified atmosphere packaged (Table 5).

Table 5: Distribution of the subtype of the fish product, type of retail outlet and packaging type of fish samples, sampled for the *L. monocytogenes* baseline survey in the EU,[(a)] 2010-2011

		No of samples	**%**
Subtype of the fish product	Unknown smoked fish	1 625	53.2
	Cold smoked fish	640	21.0
	Hot smoked fish	535	17.5
	Gravad fish	253	8.3
Type of retail outlet	Supermarket or small shop	3 004	98.4
	Other (free text field)	44	1.4
	Specialty delis	3	0.1
	Street market or farmers' market	2	0.1
Packaging type	Normal atmosphere	550	18
	Modified atmosphere	579	19
	Vacuum	1 825	59.8
	Other (free text)	99	3.2
Total		**3 053**	

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

Table 6: Distribution of the type of meat product, animal species of the origin of the meat product, possible slicing, type of retail outlet, packaging type of packaged heat-treated meat products sampled for the *L. monocytogenes* baseline survey in the EU,[(a)] 2010-2011

		No of samples	%
Type of the meat product	Cold, cooked meat product	2 547	72.2
	Sausage	780	22.1
	Pâté	203	5.8
Animal species of the origin of the meat product	Pork	2 566	72.7
	Mixed	308	8.7
	Turkey	232	6.6
	Poultry	210	5.9
	Beef	105	3.0
	Broiler	92	2.6
	Other	17	0.5
Possible slicing	Sliced	3 005	85.1
	No-Sliced	525	14.9
Type of retail outlet	Supermarket or small shop	3 466	98.2
	Other (free text field)	47	1.3
	Specialty delis	11	0.3
	Street market or farmers' market	6	0.2
Packaging type	Normal atmosphere	548	15.5
	Modified atmosphere	2 001	56.7
	Vacuum	888	25.2
	Other (free text)	93	2.6
Total		**3 530**	

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

Table 7: Distribution of subtype of cheese, type of milk treatment, animal of origin of the milk, cheese rind included in the analysis, percentage of rind, type of retail outlet and packaging type of soft or semi-soft cheeses sampled for the *L. monocytogenes* baseline survey in the EU,[(a)] 2010-2011

		No of samples	%
Subtype of cheese	Mould-ripened	1 230	35.6
	Unknown	952	27.6
	Other-wise ripened	791	22.9
	Smear-ripened	289	8.4
	Brine-matured	190	5.5
Type of milk treatment	Pasteurized milk	2 236	64.8
	Unknown	704	20.4
	Raw milk	476	13.8
	Thermised milk	36	1.0
Animal of origin of the milk	Cow	2 513	72.8
	Unknown	517	15.0
	Goat	191	5.5
	Sheep	137	4.0
	Mixed	87	2.5
	Buffalo	7	0.2
Cheese rind included in the analysis	Yes	2 411	69.8
	No	1 041	30.2

Table continued overleaf.

Table 7 (continued). Distribution of subtype of cheese, type of milk treatment, animal of origin of the milk, cheese rind included in the analysis, percentage of rind, type of retail outlet and packaging type of soft or semi-soft cheeses sampled for the *L. monocytogenes* baseline survey in the EU,[(a)] 2010-2011

		No of samples	%
Percentage of rind	< = 20 %	1 493	61.9
	Between 20 % and 40 %	714	29.6
	> = 40 %	13	0.5
	Unknown	191	7.9
Type of retail outlet	Supermarket or small shop	3 367	97.5
	Other (free text field)	55	1.6
	Specialty delis	14	0.4
	Street market or farmers' market	16	0.5
Packaging type	Normal atmosphere	2 373	68.7
	Modified atmosphere	443	12.8
	Vacuum	298	8.6
	Other (free text)	338	9.8
Total		**3 452**	

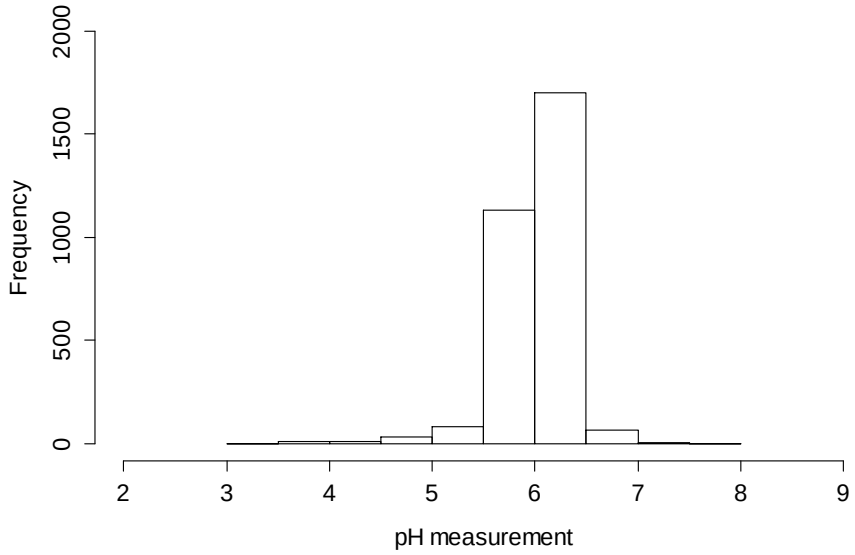
(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.

The distribution of pH and water activity measurements for fish samples at the time of sampling is shown in Table 8 and in Figures 6 and 7.

Table 8: Summary of pH and water activity measurements for packaged hot or cold smoked or gravad fish samples on the arrival at the laboratory in the EU,[(a)] 2010-2011

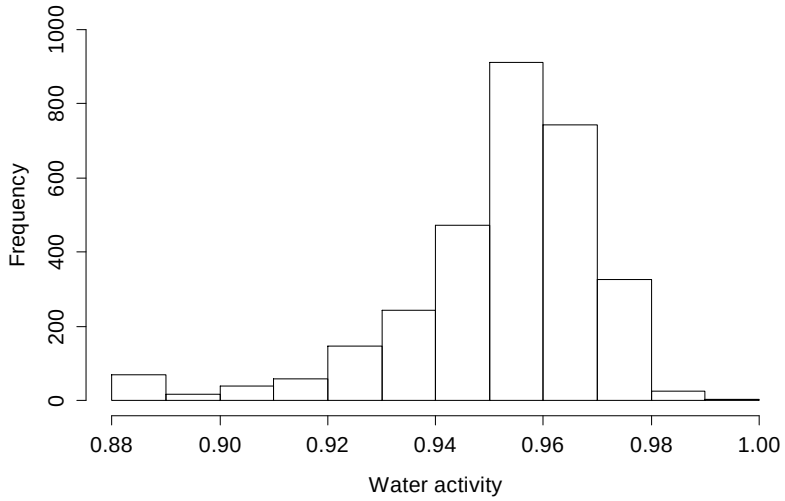
Variable	Mean	Standard Deviation
pH	6.03	0.34
Water Activity	0.96	0.02

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



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

Figure 6: Distribution of pH measurements on the arrival at the laboratory, in packaged hot or cold smoked or gravad fish samples in the EU,[(a)] 2010-2011



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

Figure 7: Distribution of water activity measurements on the arrival at the laboratory, in packaged hot or cold smoked or gravad fish samples in the EU,[(a)] 2010-2011

Based on product label information and on the criteria detailed in Appendix F, a total of 138 fish-product pairs (i.e. 138 of the fish-product batches sampled) (138/3,053 = 4.5 %) were reported as containing at least one preservative and/or acidity regulator (Table 9). Of these 138 batches, with the exception of two batches which contained three and four different preservatives and/or acidity regulators, all other fish batches contained either one or two preservatives and/or acidity regulators. The most frequently encountered combinations of preservatives and/or acidity regulators used in smoked/gravad fish products were i) sodium benzoate (E211) – reported in 37 sample pairs, ii) potassium acetate (E261) in combination with potassium lactate (E326) – reported in 36 sample pairs and c) acetic acid (E260) – reported in 29 sample pairs (detailed preservatives and/or acidity regulators data not shown).

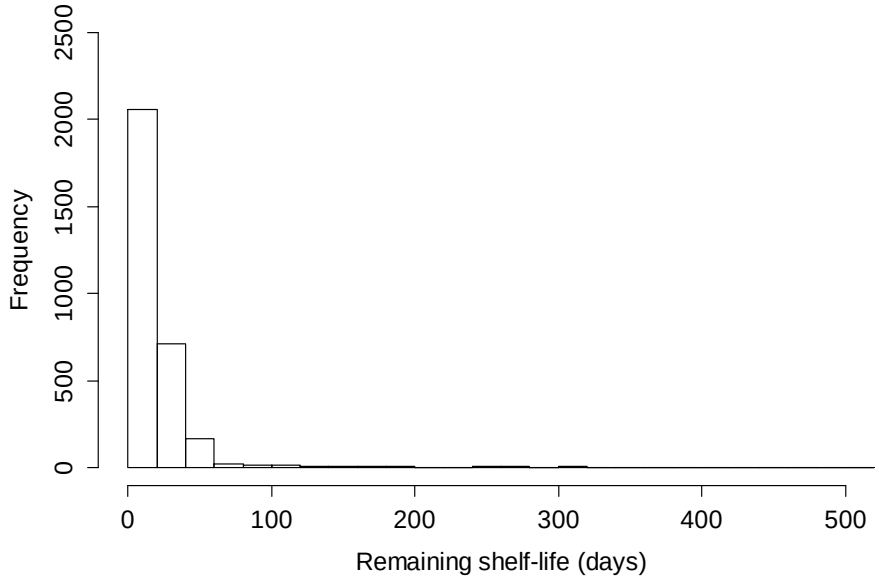
Table 9: Distribution of reported preservatives and acidity regulators in sampled packaged hot or cold smoked or gravad fish in the EU,[(a)] 2010-2011

Number of samples with specified number of preservatives and/or acidity regulators	Subtype of the fish product									
	Hot smoked	**%**	**Cold smoked**	**%**	**Gravad**	**%**	**Unknown smoked**	**%**	**Total**	**%**
0	531	99.3	601	93.9	193	76.3	1 590	97.8	2 915	95.5
1	4	0.7	8	1.3	41	16.2	30	1.8	83	2.7
2 or more	0	0	31	4.8	19	7.5	5	0.3	55	1.8
Total	535		640		253		1 625		3 053	

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

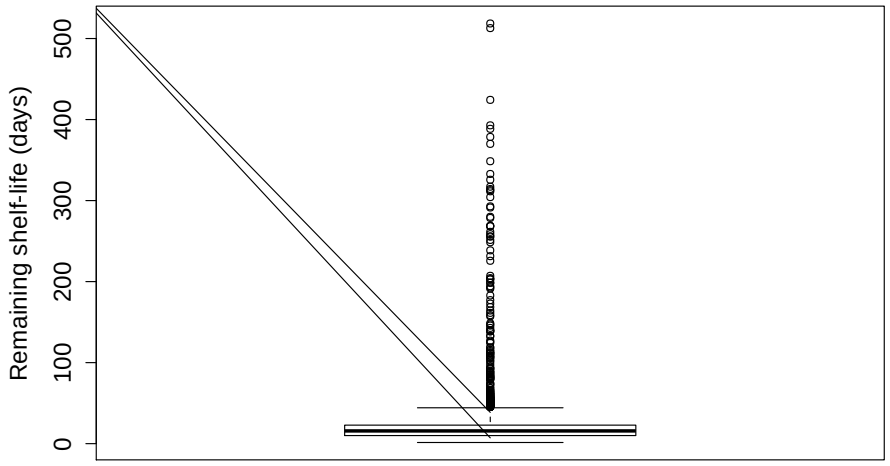
Whereas only four sampled batches of hot smoked fish (4/535 = 0.7 %) were reported as containing any preservatives and/or acidity regulators, a higher proportion of cold smoked fish products (39/640 = 6.1 %) were reported as containing one or more preservatives and/or acidity regulators. The most frequently reported combination of preservatives and/or acidity regulators in cold smoked fish products was potassium acetate (E261) with potassium lactate (E326). An even higher proportion of gravad fish product batches, almost one in four, (60/253 = 23.7 %) contained one or more preservatives and/or acidity regulators. The most frequently reported preservative in gravad fish products was acetic acid (E260). Finally, a small proportion of unknown-smoked fish batches (35/1625 = 2.2 %) contained one or two preservatives and/or acidity regulators. The most frequently reported preservative in unknown-smoked fish batches was sodium benzoate (E211) (detailed preservatives and/or acidity regulators data not shown).

The following two figures summarise the remaining shelf-life of the surveyed fish samples. This is the difference, in days, between the date of sampling and the 'Use by' date of the sampled product.



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

Figure 8: Distribution of remaining shelf-life for packaged hot or cold smoked or gravad fish samples in the EU,[(a)] 2010-2011



(a): The lower whisker represent the lowest value, bottom of the box represents the first quartile of the distribution and the top the third quartile, whereas the bar inside the box represents the median. The upper whisker represents the maximum value or 1.5 times the difference between the third and the first quartile (interquartile range). Small circular symbols indicate extreme values, with a value larger than the upper whisker (217 extreme values).
(b): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.

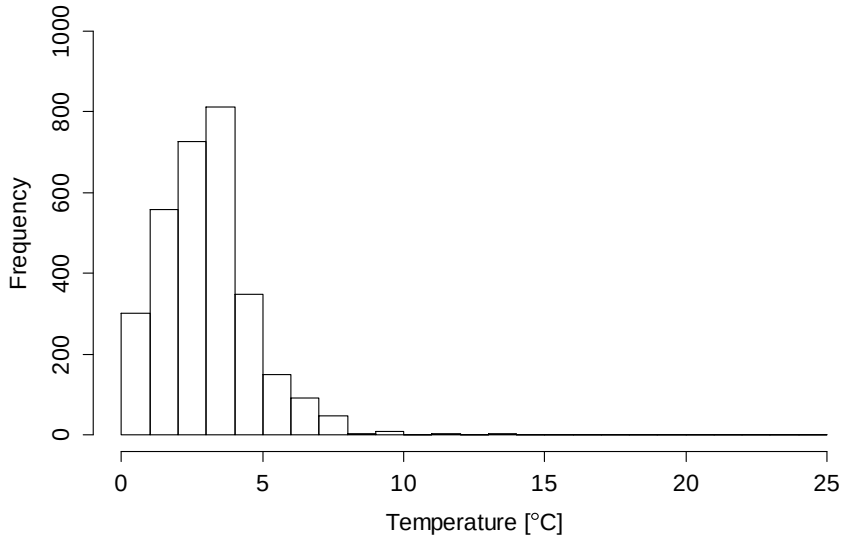
Figure 9: Box plot[(a)] of remaining shelf-life for packaged hot or cold smoked or gravad fish samples in the EU,[(b)] 2010-2011

The storage temperatures at retail and at the laboratory up to the end of shelf-life for surveyed food samples are summarised in Figures 10-15 and in Table 10.

Table 10: Summary of storage temperature at retail and at laboratory up to the end of shelf-life for packaged hot or cold smoked or gravad fish, packaged heat-treated meat products and soft or semi-soft cheese samples of the *L. monocytogenes* baseline survey in the EU,[(a)] 2010-2011

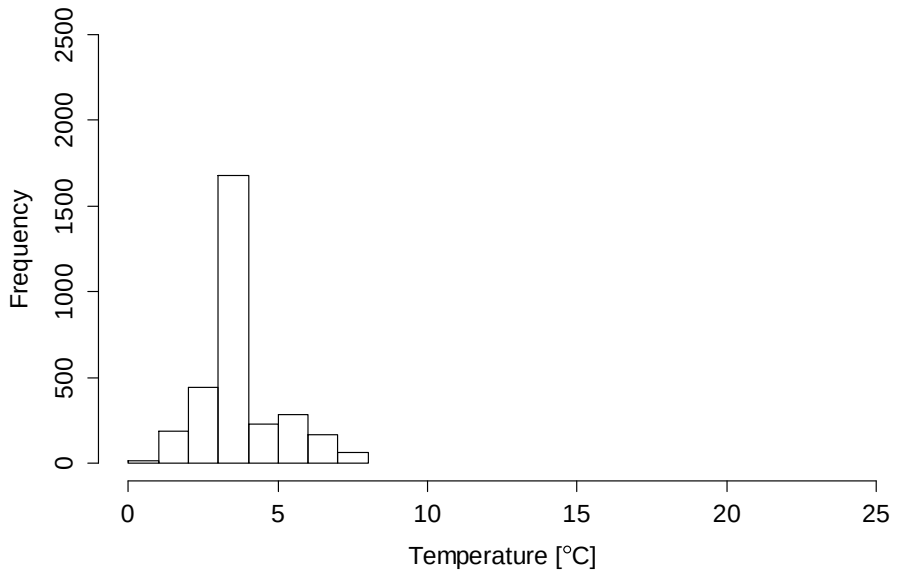
Product	Retail Storage (sample surface)		Laboratory Storage	
	Mean	Standard Deviation	Mean	Standard Deviation
Packaged hot or cold smoked or gravad fish	3.45	1.79	4.22	1.28
Packaged heat-treated meat products	3.71	1.78	4.51	1.43
Soft or semi-soft cheeses	4.09	1.83	5.06	1.92

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



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

Figure 10: Distribution of storage temperature at retail (sample surface temperature) for packaged hot or cold smoked or gravad fish samples in the EU,[(a)] 2010-2011



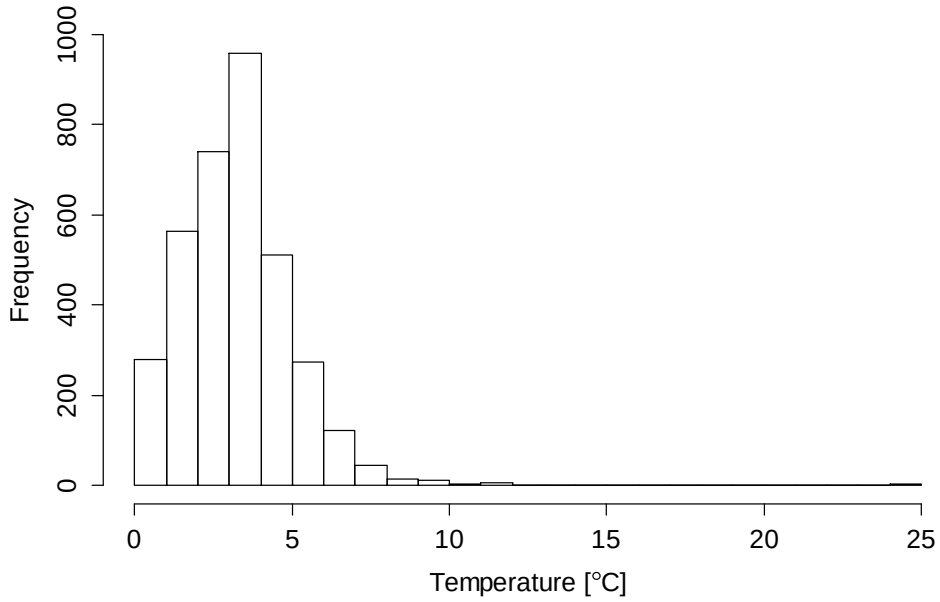
(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.

Figure 11: Distribution of storage temperature at laboratory up to the end of shelf-life for packaged hot or cold smoked or gravad fish samples in the EU,[(a)] 2010-2011

Table 11: Distribution of packaging type by slicing for packaged heat-treated meat products sampled for the *L. monocytogenes* baseline survey in the EU,[(a)] 2010-2011

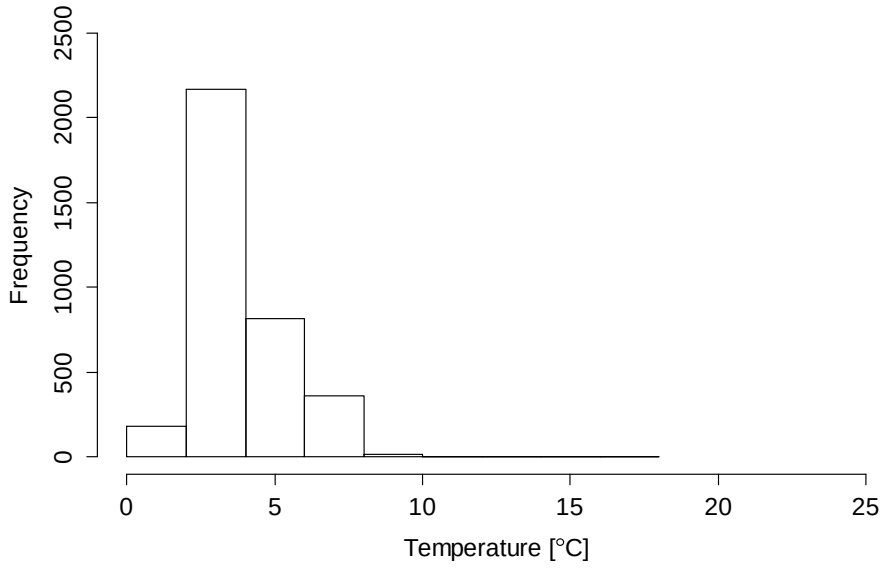
Packaging type	Sliced		Not Sliced		Total	
	N	%	N	%	N	%
Modified atmosphere	1 916	63.8	85	16.2	2 001	56.7
Vacuum	586	19.5	302	57.5	888	25.2
Normal atmosphere	457	15.2	91	17.3	548	15.5
Other (free text)	46	1.5	47	9.0	93	2.6
Total	3 005		525		3 530	

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



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

Figure 12: Distribution of storage temperature at retail (sample surface temperature) for packaged heat-treated meat products sampled for the *L. monocytogenes* baseline survey in the EU,[(a)] 2010-2011



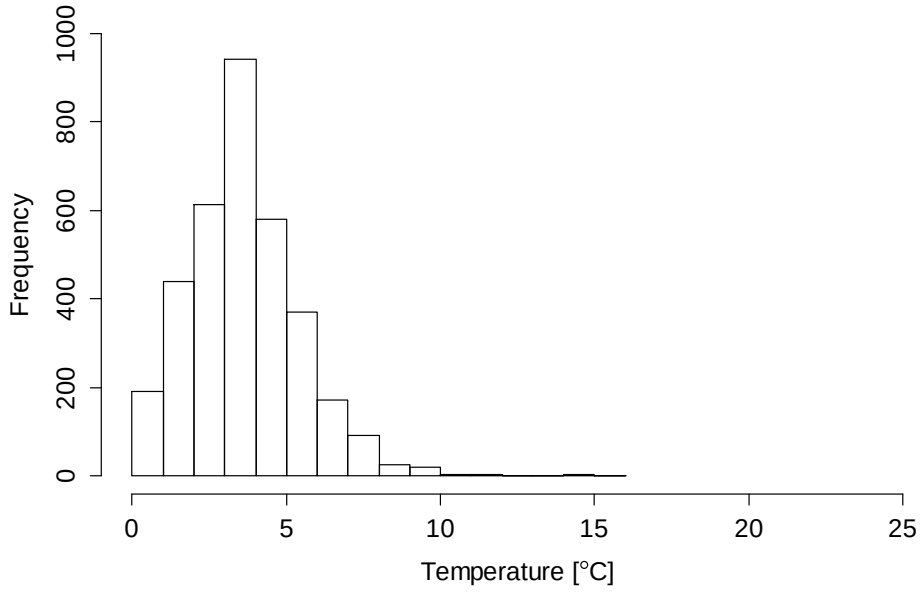
(a): Portugal did not participate in the baseline survey and one non-MS, Norway, participated. The Norwegian samples are included in this presentation.

Figure 13: Distribution of storage temperature at laboratory up to the end of shelf-life for packaged heat-treated meat product samples in the EU,[(a)] 2010-2011

Table 12: Distribution of subtype of cheese by type of milk treatment for soft or semi-soft cheeses sampled for the *L. monocytogenes* baseline survey in the EU,[(a)] 2010-2011

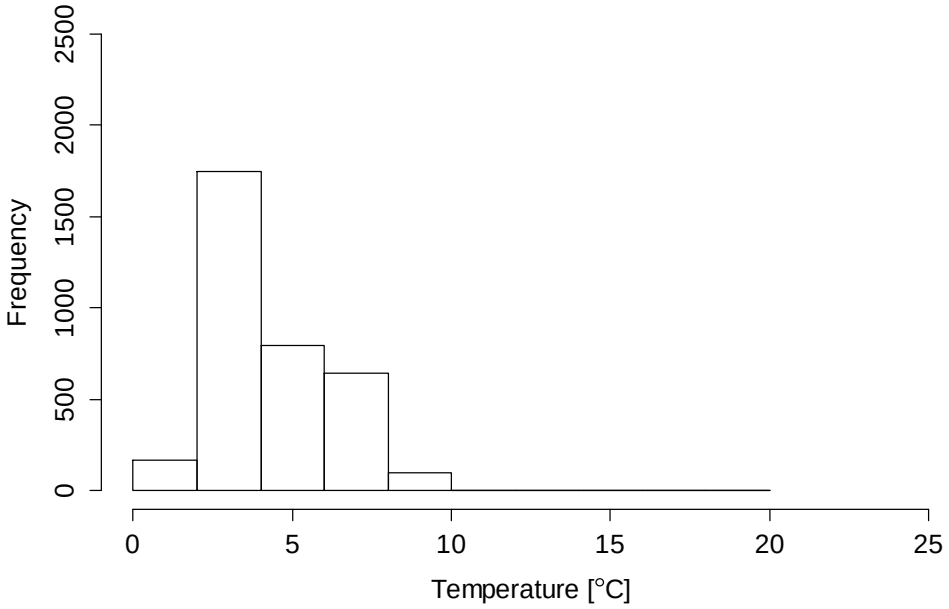
Subtype of cheese	Type of milk treatment				
	Pasteurized milk	**Raw milk**	**Thermised milk**	**Unknown**	**Total**
Brine-matured	167	6	0	17	190
Mould-ripened	837	195	12	186	1 230
Other-wise ripened	621	102	10	58	791
Smear-ripened	197	67	4	21	289
Unknown	414	106	10	422	952
Total	2 236	476	36	704	3 452

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



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

Figure 14: Distribution of storage temperature at retail (sample surface temperature) for soft or semi-soft cheeses sampled for the *L. monocytogenes* baseline survey in the EU,[(a)] 2010-2011



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

Figure 15: Distribution of storage temperature at laboratory up to the end of shelf-life for soft or semi-soft cheeses sampled for the *L. monocytogenes* baseline survey in the EU,[(a)] 2010-2011

Appendix F. Categorization of sampled fish products according to reported food additives

As part of the sampling and recording procedures outlined in Commission Decision 2010/678/EU, MSs had been asked to record and report on 'preservatives used in smoked or gravad fish (as indicated on the label)'. It should be noted that, in addition to reporting on preservatives, the EFSA report on the proposed technical specifications for the survey also recommended reporting of acidity regulators.

In the resulting dataset, many different combinations of food additives and other ingredients (i.e. not merely 'preservatives' *per se*) were recorded. Since input was based solely on product label information, the concentration of the reported food additives was unknown in almost all cases. For instance, with the exception of three sampled batches for which quantitative data were present for sodium chloride ("2-3 % NaCl"), in all other cases only the names of the food additives listed on the labels of sampled fish products were available/recorded. In addition, it is reasonable to assume that in some of the sampled batches, although common fish additives such as smoke and salt might have been added by the manufacturer, they may not have been reported as such (i.e. not included in the product label). It is also possible that sampling officials did not report labelled constituents/ingredients that were considered not to constitute preservatives.

Some of the additives reported in smoked and gravad fish can have multiple functions in foods and be used for different (primary) purposes in different food matrices. For instance, although Annex I of Council Directive 89/107/EEC on the approximation of the laws of the Member States concerning food additives authorized for use in foodstuffs intended for human consumption[23] provides a list of 24 categories of food additives, a given food additive (e.g. sodium lactate) can serve both as an acidity regulator (i.e. via lowering of the pH of food) as well as a preservative (i.e., contributing to food preservation via the antimicrobial action of its undissociated form).

Most preservatives are, in essence, antimicrobials, added to prevent or delay the microbiological spoilage, or promote the microbiological safety of foods. However, other types of preservatives prolong food shelf-life by inhibiting or delaying chemically-induced food deterioration (e.g. some antioxidants). In addition, upon their concurrent addition in foods, even under moderate concentrations, several food additives are known to exert synergistic antimicrobial effects under the 'hurdles concept'. It is well known that the antimicrobial efficacy of food additives possessing antimicrobial activity is dependent on their concentration, matrix of application (e.g. laboratory broths *vs*. actual foods) and food storage conditions including the type of food packaging. The lack of quantitative data on fish additives along with the reasons outlined above hinders the ability to classify the tested fish samples into distinctive and mutually exclusive categories based on the type of food additive(s) used. Hence, the available information is not optimal for a thorough analysis of the possible effects of fish additives on *L. monocytogenes* in the examined samples. Forty seven different combinations of food additives and ingredients other than fish flesh were reported for the 3 053 pairs of fish products sampled in this survey. No food additive was reported for the majority (1 922 pairs, 63 %) of the fish sample pairs. The food additive status of a considerable proportion of fish sample pairs were reported as "unknown" (459 pairs, 15 %) whereas "other" was the reported input for small proportion of sample pairs (45 samples, 1.5 %).

Some of the reported additives and listed ingredients were excluded from the analysis (sunflower oil, food colorants (E110 and E124), ascorbic acid (E300), calcium di-phosphate (E450) and mono-sodium glutamate (E621)). In addition, the classification used in this Report did not take into consideration the addition of sodium chloride, potassium chloride, sugar, smoke, herbs and spices (i.e. compounds that are known, based on the scientific literature, as having the potential to provisionally exert antimicrobial action or contribute (directly or indirectly) towards food preservation, but, with the exception of potassium chloride (E508), are not assigned an E-number. The eight groups of reported

[23] Council Directive 89/107/EEC of 21 December 1988 on the approximation of the laws of the Member States concerning food additives authorized for use in foodstuffs intended for human consumption. OJ L 40, 11.2.1989, p. 27-33.

preservatives and/or acidity regulators used for classification were sorbic acid and its salts (E200, E202, E203), benzoic acid and its salts (E210, E211, E212), sodium nitrite (E250), acetic acid and its salts (E260, E261, E262, E263), lactic acid and its salts (E270, E325, E326, E327), citric acid (E330), penta-sodium-triphosphate (E451i) and glucono-δ-lactone (E575).

Appendix G. Comparison of results of detection and enumeration tests for surveyed food samples

Appendix G.1. Comparison of results of detection and enumeration tests for packaged smoked or gravad fish samples

The results of the detection test and of the enumeration test for each of the surveyed fish samples were cross-classified, in Table 13, for testing at time of sampling and in Table 14, for testing at the end of shelf-life. It is interesting to note that 79.4 % (247 samples out of 311) and 69.8 % (213 samples out of 305) of the detection-positive samples (at sampling and at the end of shelf-life, respectively) were negative by the enumeration test. Conversely, 3.0 % (2 samples out of 66) and 7.1 % (7 samples out of 99) of the samples that were positive by enumeration at sampling and at end of shelf-life, respectively, were detection-test negative.

Table 13: Classification of packaged hot or cold smoked or gravad fish samples based on the results of *L. monocytogenes* detection and enumeration testing at sampling, in the EU,[(a)] 2010-2011.

Detection Testing	Enumeration testing: at least 10 cfu/g		
	Negative	**Positive**	**Total**
Negative	2 740	2	2 742
Positive	247	64	311
Total	2 987	66	3 053

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

Table 14: Classification of packaged hot or cold smoked or gravad fish samples based on the results of *L. monocytogenes* detection and enumeration testing at end of shelf-life, in the EU,[(a)] 2010-2011.

Detection Testing	Enumeration testing: at least 10 cfu/g		
	Negative	**Positive**	**Total**
Negative	2 741	7	2 748
Positive	213	92	305
Total	2 954	99	3 053

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

For more details, the reader is referred to Tables 87 and 89 of the External Report (Rakhmawati et al., 2013).

Appendix G.2. Comparison of results of detection and enumeration tests for packaged heat-treated meat products

The results of the detection test and of the enumeration test for each of the surveyed meat samples were cross-classified, in Table 15. It is interesting to note that 56.3 % (40 samples out of 71) of the detection-positive samples were negative by the enumeration test. Conversely, 3.1 % (1 sample out of 32) of the samples that were positive by enumeration were detection-test negative.

Table 15: Classification of packaged heat-treated meat product samples based on the results of detection and enumeration testing at the end of shelf-life, in the EU,[(a)] 2010-2011.

Detection Testing	Enumeration testing: at least 10 cfu/g		
	Negative	**Positive**	**Total**
Negative	3 458	1	3 459
Positive	40	31	71
Total	3 498	32	3 530

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

For more details, the reader is referred to Table 91 of the External Report (Rakhmawati et al., 2013).

Appendix H. Comparison of results of detection and enumeration tests for soft or semi-soft cheese samples

The results of the detection test and of the enumeration test for each of the surveyed cheese samples were cross-classified, in Table 16. It is interesting to note that 80 % (12 samples out of 15) of the detection-positive samples were negative by the enumeration test. Conversely, 25 % (1 sample out of 4) of the samples that were positive by enumeration were detection-test negative.

Table 16: Classification of soft or semi-soft cheese samples based on the results of *L. monocytogenes* detection and enumeration testing at the end of shelf-life, in the EU,[(a)] 2010-2011.

Detection Testing	Enumeration testing: at least 10 cfu/g		
	Negative	**Positive**	**Total**
Negative	3 436	1	3 437
Positive	12	3	15
Total	3 448	4	3 452

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

For more details, the reader is referred to Table 93 of the External Report (Rakhmawati et al., 2013).

ABBREVIATIONS

a_w	Water activity
cfu	Colony forming units
CI	Confidence Interval
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EU	European Union
GEE	Generalized Estimating Equations
ISO	International Organization for Standardization
MSs	Member State(s)
NRL	National Reference Laboratory
pH	p[H], often written as, pH, is a measure of hydrogen ion concentration; a measure of the acidity or alkalinity of a solution.