SCIENTIFIC REPORT OF EFSA

Analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses, in the EU, 2008[1]

Part B: Analysis of factors associated with *Salmonella* contamination of broiler carcasses

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ABSTRACT

A European Union-wide baseline survey on *Campylobacter* in broiler batches and on *Campylobacter* and *Salmonella* on broiler carcasses was carried out in 2008. In the *Salmonella* sub-survey a total of 10,035 broiler batches were sampled from 561 slaughterhouses in 26 European Union Member States and two countries not belonging to the European Union. From each randomly selected batch one carcass was collected after chilling and the neck skin together with the breast skin was examined for the presence of *Salmonella*. Multivariable regression analysis showed that the risk for *Salmonella*-contaminated carcasses increased with the slaughter capacity of the slaughterhouse and with processing of the carcass later during the day. The risk for contamination of carcasses with *Salmonella* varied significantly between countries and between slaughterhouses within a country, even when other associated factors were accounted for. The *Salmonella* serovar distribution varied among Member States, many of them having a specific distribution pattern of their own and no specific serovar was predominant in all countries in the survey. The most commonly reported serovars were *S.* Infantis, *S.* Enteritidis and *S.* Typhimurium. Many of the reported serovars seem to have become well-established in broiler production.

KEY WORDS

Salmonella, broiler carcasses, chicken, baseline survey, risk factors, EU.

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SUMMARY

A European Union-wide baseline survey on *Campylobacter* in broiler batches and on *Campylobacter* and *Salmonella* on broiler carcasses was carried out in 2008. In the *Salmonella* subsurvey, a total of 10,035 broiler batches were sampled from 561 slaughterhouses in 26 European Union Member States, plus Norway and Switzerland. From each randomly selected batch, one carcass was collected after chilling and the neck skin together with the breast skin was examined for the presence of *Salmonella* and *Campylobacter*. The results of the analysis of *Salmonella* prevalence have already been published by the European Food Safety Authority on 17 March 2010 in the Part A report. The Part B report on the *Campylobacter* subsurvey was published by the European Food Safety Authority on 5 August 2010. The present Part B report on the *Salmonella* subsurvey provides the results from analyses of the associations of eight-batch or slaughterhouse level factors and *Salmonella* contamination of carcasses. The investigated prevalence was the observed prevalence, meaning that the prevalence estimates did not account for imperfect test characteristics.

Multivariable regression analysis showed that the risk for *Salmonella*-contaminated carcasses increased with the slaughter capacity of the slaughterhouse and with processing of the carcass later during the day. The risks for contamination of carcasses with *Salmonella* varied significantly between countries and between slaughterhouses within a country, even when other associated factors were accounted for. The results showed that for the country group having a lower *Salmonella* prevalence other factors were associated with *Salmonella* contamination of broiler carcasses. Specifically, for the group of countries with prevalence below the European Union[4] median, the only factor indicating an association with *Salmonella*-contaminated carcasses was the type of chilling used for the carcasses, as the risk of *Salmonella* contamination of carcasses appeared to be lower if broiler carcasses were chilled by a mixed-chilling method. This factor was not significant in the analysis based on the overall dataset nor in the analysis of the subset of group of countries with prevalence above the European Union median. For this latter group of countries the associated factors were consistent with the results based on the overall European Union level dataset.

Factors that were included in the analysis, but which were not significantly associated with *Salmonella* contamination of carcasses were flock production type, thinning of flocks, age of broilers, quarter of sampling during the year, time between sampling and testing, the carcass chilling method used and *Campylobacter* contamination results on the broiler carcass. For some of the factors tested, the power of the analyses was low due to too few samples in some specific categories. Moreover, the analyses showed that 46% of the unexplained variance in the *Salmonella* contamination results might have been attributable to slaughterhouse-specific factors for which no data were gathered during the survey.

The *Salmonella* serovar distribution varied among Member States, many of them having a specific distribution pattern of their own and no specific serovar was predominant in all countries in the survey. The most commonly reported serovars were *S.* Infantis, *S.* Enteritidis and *S.* Typhimurium. Although there was a concentration of most *S.* Infantis isolates in one Member State it was the most widely-distributed serovar and reported by 15 countries. This indicates that the presence of *S.* Infantis is not a local phenomenon. *S.* Enteritidis was present in 14 countries and the dominant serovar in five countries confirming its role as the most important serovar found in broilers in Europe. *S.* Typhimurium was less frequently reported compared to *S.* Kentucky but was more spread across Europe. *S.* Agona and *S.* Mbandaka were also widely distributed, although at a lower prevalence. The serovar distribution in broiler carcasses tended generally to be the same and in similar proportions as the distribution observed in the broiler flocks baseline survey, even though the latter survey had been conducted two years earlier. This suggested that many of the serovars have become well-established in the broiler production. The descriptive analysis of the serovar distribution also supported the notion that broiler meat contributes to human *Salmonella* infection.

[4] Two non-MSs, Norway and Switzerland, were included in the overall EU level dataset.

Based on the prevalence of *Salmonella*-contaminated broiler carcasses, a simulation exercise was performed to investigate the Member State-specific probability of meeting the *Salmonella* process hygiene criteria in poultry meat as laid down by Regulation (EC) No 2073/2005. The outcome of this simulation exercise was rather uncertain for about one third of the Member States. Of the remaining group of countries, five Member States and Norway and Switzerland would meet those *Salmonella* microbiological process hygiene criteria in poultry meat.

It is recommended that Member States consider the factors found to be associated with *Salmonella*-contaminated broiler carcasses at European Union level in this survey, when they are designing and implementing national *Salmonella* control programmes for broiler meat. Member States are specifically encouraged to verify the food business operators' own controls for *Salmonella* in their slaughterhouses in order to prevent subsequent contamination of broiler carcasses and to improve the protection of public health. Further national studies identifying more closely the factors that put broiler carcasses at risk of becoming contaminated with *Salmonella* in a country are recommended, taking into account the national *Salmonella* prevalence and the characteristics of the national broiler production, including slaughter procedures.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

BACKGROUND

Regulation (EC) No 2160/2003[5] on the control of *Salmonella* spp. and other specified zoonotic agents provides for the setting of Community targets for reducing the prevalence of *Salmonella* serovars with public health significance in food or animal populations.

Upon a request from the European Commission, the European Food Safety Authority (EFSA) issued a Report of the Task Force on Zoonoses Data Collection on proposed technical specifications for a co-ordinated monitoring programme for *Salmonella* and *Campylobacter* in broiler meat in the European Union (EU) (EFSA, 2007a). Previously, a Commission Task Force of scientific experts in collaboration with EFSA prepared technical specifications for a baseline study on a harmonised monitoring of *Campylobacter* in broiler flocks.

Based on EFSA's proposal and the Commission's technical specifications, the Commission adopted the Decision 2007/516/EC[6] of 19 July 2007 concerning a financial contribution from the Community towards a survey on the prevalence and antimicrobial resistance of *Campylobacter* spp. in broiler flocks and on the prevalence of *Campylobacter* spp. and *Salmonella* spp. on broiler carcasses to be carried out in Member States (MSs). This large survey, consisting of two subsurveys, was carried out by the EU MSs during the period 1 January 2008 to 31 December 2008.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission requested EFSA, on 2 April 2008, to analyse the results of the baseline survey on *Campylobacter* spp. in broiler flocks and on *Campylobacter* spp. and *Salmonella* spp. in broiler carcasses, in particular:

- to estimate the prevalence of *Campylobacter* spp. in broiler flocks and the prevalence of *Campylobacter* spp. and *Salmonella* spp. on broiler carcasses in MSs and at the level of the European Union; and
- to assess quantitatively the risk factors for *Campylobacter* spp. in broiler flocks and *Campylobacter* spp. and *Salmonella* spp. on broiler carcasses based on the information collected.

5 Regulation (EC) No. 2160/2003 of the European Parliament and of the Council of 17 November 2003 on the control of *Salmonella* and other specified food-borne zoonotic agents, OJ L 325, 12.12.2003, p.1.

6 Commission Decision 2007/516/EC of 19 July 2007 concerning a financial contribution from the Community towards a survey on the prevalence and antimicrobial resistance of *Campylobacter* spp. in broiler flocks and on the prevalence of *Campylobacter* spp. and *Salmonella* spp. in broiler carcasses to be carried out in the Member States. OJ L 190, 21.07.2007, p. 25.

ANALYSIS

1. Introduction

A baseline survey (BS) was carried out in the EU to estimate the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses at slaughterhouse level. This study was the sixth in a series of BSs carried out within the EU and it was the first BS directly investigating foodstuffs. The objective of the survey has been to obtain comparable data for all MSs through harmonised sampling schemes. According to Article 5 of Directive 2003/99/EC[7] of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, 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 at MS level. Results of such a survey will inform of the need for an EU-wide intervention.

A scientific report by EFSA on the "Analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses, in the EU, 2008, Part A: *Campylobacter* and *Salmonella* prevalence estimates" (EFSA, 2010a) was published on 17 March 2010. This Part A report included the estimation of the prevalence of *Campylobacter*-colonised broiler batches, of *Campylobacter*-contaminated broiler carcasses and of *Salmonella*-contaminated broiler carcasses at EU level and for each MS; the analyses of the *Campylobacter* enumeration results on broiler carcasses as well as the analyses of the most frequently identified *Campylobacter* species in broiler batches and *Campylobacter* species and *Salmonella* serovars on broiler carcasses.

Two Part B reports were produced regarding this BS. A first report part B was published on 5 August 2010 and provided the EU level analyses of factors associated with *Campylobacter*-colonised broiler batches and/or with *Campylobacter*-contaminated broiler carcasses, further analyses of the identified *Campylobacter* species distribution across the EU, as well as the results of the investigation of the diagnostic sensitivity of the detection method applied to estimate the prevalence of *Campylobacter*-contaminated broiler carcasses. The second, present, Part B report presents the analyses of factors associated with *Salmonella*-contaminated broiler carcasses as well as more in-depth analyses of the identified *Salmonella* serovar distributions. In addition, results are cited from a scientific report entitled "Simulation-based assessment of microbiological criteria on *Salmonella* in poultry meat" from EFSA's Assessment Methodology Unit (EFSA, 2011) wherein the rationale, methodology, results and discussion for this report can be found. The results of the antimicrobial resistance of the *Campylobacter* and *Salmonella* isolates were evaluated, in accordance with Article 9 of Directive 2003/99/EC, in the annual report on antimicrobial resistance in the EU in 2008 (EFSA, 2010b).

The slaughterhouse survey was carried out over a one-year period, starting in January 2008. Sampling was based on a random selection, both regarding slaughterhouses, sampling days each month and which batches are to be sampled on a selected day.

The objectives, sampling frame and methods of bacteriological analysis, as well as the collection and reporting of data, and the timelines of this BS were specified in Commission Decision 2007/516/EC.

Twenty-six EU MSs participated in the survey. Greece did not carry out the survey. In addition, two countries not belonging to the EU, Norway and Switzerland (hereafter referred to as non-MSs), participated in the survey.

2. Definitions

In the scope of this report the following definitions are used:

7 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/EC and repealing Council Directive 92/117/EC. OJ L 325, 12.12.2003 p. 31.

Broiler: a male or female chicken raised specifically for meat production and intended for slaughter.

Broiler batch: a group (or batch) of broilers, which have been raised in the same flock and which are delivered and slaughtered on one single day.

Broiler carcass: the body (or carcass) of a broiler collected after slaughter, dressing (plucking and removal of the offal), and immediately after chilling, but before any further processing, such as freezing, cutting or packaging.

***Campylobacter*:** all *Campylobacter* spp. which can be isolated by the prescribed culture techniques. These techniques include incubation at 42°C, hence the term thermophilic *Campylobacter*(s) is commonly used.

***Campylobacter*- and/or *Salmonella*-contaminated carcass:** a broiler carcass from which *Campylobacter* spp. and/or *Salmonella* spp. was isolated.

(Diagnostic) sensitivity: means the conditional probability that a *Salmonella*-contaminated carcass will be positive for the prescribed survey culture technique.

(Diagnostic) specificity: means the conditional probability that a *Salmonella*-non-contaminated carcass will be negative for the prescribed survey culture technique.

Prevalence: means the observed (apparent) prevalence estimate that accounts for the aspects of clustering and of weighting but not for imperfect (test) sensitivity or specificity.

Proportion (%) of positive units: means the number of positive units out of the sampled units and does not account for any design aspect, such as clustering.

***Salmonella*:** all *Salmonella* spp. which can be isolated by the prescribed culture technique.

3. Objectives

The specific objectives related to this second Part B report were:

- to investigate the effects of factors, which may be associated with *Salmonella*-contaminated broiler carcasses, at EU level;
- to investigate the slaughterhouse-specific effects on *Salmonella*-contaminated broiler carcasses;
- to investigate the *Salmonella* serovar distribution and determine the most frequently occurring *Salmonella* serovars on broiler carcasses across the EU; and
- to investigate the MS-specific probability of meeting the *Salmonella* microbiological process hygiene criteria in poultry meat as laid down by Regulation (EC) No 2073/2005 based on the prevalence of *Salmonella*-contaminated broiler carcasses and *vice versa*.

4. Materials and Methods

A detailed description of the design of the BS, sample design, sample sizes and bacteriological analyses is found in Commission Decision 2007/516/EC and in the Part A report. Aspects of the survey design, laboratory analysis, and data of particular relevance to data analysis and interpretation are described here.

4.1. Survey design

The survey took place in the EU between January and December 2008 and was conducted at broiler-batch level in slaughterhouses, focusing on birds entering the food chain[8]. The sampling of broiler batches was based on a random selection of slaughterhouses, sampling days in each month and the batches to be sampled on each sampling day. The randomisation scheme aimed at selecting broiler batches proportionate to the number of broiler flocks, fattened according to the different production types (conventional, free-range or organic), and avoiding the introduction of biases due to the potential knowledge of the infection status of the holding from which the broiler batch originated. In addition, MSs were asked to stratify sampling to ensure an even spread throughout the survey period in order to investigate seasonal effects on the outcomes.

From each randomly selected batch, one whole carcass was collected immediately after chilling but before freezing, cutting or packaging, for the detection of *Salmonella* and for the detection and enumeration (determination of counts) of *Campylobacter*. At the laboratory, the neck skin was removed, if present, together with the skin from one side of the carcass (breast skin) avoiding any fat, to make a test portion.

Isolation of *Salmonella* organisms on the broiler carcass samples was undertaken as described by the International Organization for Standardization (ISO) in ISO 6579:2002(E) 'Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp.'. At least one *Salmonella* isolate per positive batch was identified using phenotypic detection methods as described in ISO 6579:2002(E) and serotyped. Detection and enumeration of *Campylobacter* were performed using the same initial test portion from each sampled carcass.

Sampling management, laboratory analysis and data submission were carried out by the competent authorities of the MS or under their supervision.

4.2. Data description

A detailed description of the validation and cleaning of the dataset carried out was provided in the Part A report. The final cleaned dataset for the survey on *Salmonella* on broiler carcases contained data from 10,035 broiler batches sampled from 561 slaughterhouses in 26 MSs and two non-MSs.

4.3. Analysis of factors associated with *Salmonella*-contaminated broiler carcasses

The general assumptions and framework of the statistical analysis carried out are reported in detail in the Part A report. The effects of factors potentially associated with *Salmonella* contamination were analysed at carcass level. Factors were investigated for any association with the EU level prevalence, meaning that the prevalence estimates accounted for the aspects of clustering and of weighting but not for imperfect test characteristics. The EU level prevalence of *Salmonella*-contaminated broiler batches was defined as the prevalence of positive carcasses processed over the one-year period of the BS, at EU level. In the analysis for this Part B report, Norway and Switzerland are included in the EU level dataset.

4.3.1. Definition of the outcome variables

The outcome variable that was considered was *Salmonella* spp. contamination of broiler carcasses based on detection method, as a binary outcome variable (positive/negative). In the Part A report, prevalence of *Salmonella* spp. *(Salmonella)*; *Salmonella* Enteritidis (*S.* Enteritidis) and/or *Salmonella* Typhimurium (*S.* Typhimurium); and serovars other than *Salmonella* Enteritidis or Typhimurium were presented. However, *S.* Enteritidis and *S.* Typhimurium were relatively infrequent and only detected

8 Sampling appeared to be evenly distributed over the year for most of the participating countries, even though some MSs (Italy, Latvia, Luxembourg, Malta, Portugal and Romania) did not collect samples during one to up to eight months in 2008.

on broiler carcasses in 17 MSs and in one non-MS, resulting in an EU prevalence of 3.6%. Therefore, the analysis of associated factors for the specific outcome of *S.* Enteritidis and/or *S.* Typhimurium positivity was not carried out due to a low power of analysis.

4.3.2. Factors investigated

Data on factors potentially associated with the above-mentioned outcome was collected using a mandatory questionnaire by the competent authorities, or under their supervision, at the time of sampling in the slaughterhouses. The relevant factors are listed in Table 1 and are described in detail in Appendix B. Some additional (optional) data and variables were collected on a voluntary basis by MSs. However, the effects of these optional factors could not be evaluated due to the scarcity of data reported.

Table 1: Factors collected by a questionnaire and potentially associated with *Salmonella*-contaminated broiler carcasses, from the baseline survey in the EU [(a)], 2008

Factors
Flock production type (*conventional, free-range standard, free-range organic, unknown* [(b)])
Previous thinning of the flock (*yes, no, unknown*)
Age of broilers (*days*)
Date of sampling [(c)]
Time (*hour*) of sampling during the day
Time (*hours*) between sampling and testing [(d)]
Capacity of slaughterhouse (*number of broilers slaughtered per year in the slaughterhouse*)
Type of chilling of carcasses (*air, immersion, spray*)
Campylobacter contamination result on the broiler carcass

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated.
(b): In a conventional flock type, birds are housed. A free-range flock system is a flock production type where birds have outdoor access. An organic flock system is a production type that is similar to the free-range system and that fulfils the requirements set out for organic production; birds have outdoor access and are registered with a recognised organic standard regulatory organisation.
(c): Recoded into a new variable 'quarter of sampling'.
(d): Factor related to the sensitivity of the sampling and testing process.

During the data analyses, certain decisions were made regarding (re-)coding and the use of recorded factors. Firstly, the variable 'date of sampling' was recoded into a new variable 'quarter of sampling' as follows: first quarter: January to March; second quarter: April to June; third quarter: July to September; and fourth quarter: October to December 2008. Secondly, the age of broilers was considered using a scale of 10 days to assess the risk of *Salmonella* contamination by 10-day increments. Finally, in the list of factors, some categorical variables were included which have a natural ordering, such as 'capacity of slaughterhouses'. Some combinations of classes of these categorical variables were characterised by sparse or no observations, resulting in a problematic fit of regression models. Rather than treating them as nominal, categorical variables, they were used as continuous variables, after having been assigned a score, corresponding to the estimated midpoint of each category, to reflect the distance between each category. This approach is illustrated in Table 2 for the factor 'capacity of slaughterhouses'. The parameter estimates corresponding to the scores were mainly used to assess the direction of the effect, without giving too much emphasis (interpretation) on the size of the effect.

The factor 'time in hours between sampling and testing' was considered to influence potentially the sensitivity of the testing process[9] but not a potential risk factor per se. Therefore, when this factor was retained in final regression models, its results were not shown.

Table 2: Description of scores for slaughterhouse capacity

Capacity of the slaughterhouse	Midpoint	Log_{10} of midpoint	Scores to be used in the models
< 100,000	50,000	4.70	4.7
100,000-499,999	300,000	5.48	5.5
500,000-999,999	750,000	5.87	5.9
1,000,000-4,999,999	3,000,000	6.48	6.5
5,000,000-9,999,999	7,500,000	6.87	6.9
≥ 10,000,000	15,000,000	7.18	7.2

4.3.3. Exploratory bivariable analysis of potentially associated factors

A thorough description was made of the samples by all recorded factors or variables. Categorical variables were analysed through frequency tables and bar graphs. Multiple bar graphs, by MS and for the global EU dataset, were produced by lattice packages in the R software. Quantitative variables were described through measures of central tendency and dispersion such as mean and standard deviation as well as median and first and third quartiles. Boxplots were used for graphical visualisation.

The association between each potentially associated factor and the outcome variable was visually presented by:

a) multiple bar graphs of estimated frequency counts of *Salmonella*-positive and -negative broiler carcasses, by MS and different levels of categorical variables;

b) bar graphs of (weighted) prevalence and 95% confidence intervals (CIs), by different levels of categorical variables; and

c) boxplots of quantitative variables for *Salmonella*-positive and -negative broiler carcasses.

The association between each factor and the outcomes of interest were tested separately by Chi-square tests, Spearman correlation and Cochran-Mantel-Haenszel Chi-square tests for linear trends. Due to possible confounding[10] and interaction, these results should be interpreted cautiously and only within the context of an exploratory analysis.

4.3.4. Identification of factors associated with *Salmonella*-contaminated broiler carcasses

Multivariable regression analysis was applied to obtain adjusted estimates of the effect of factors associated with the outcome of interest. The inclusion of multiple factors (predictors) in a regression model allows the adjustment for confounding that may result from association among these factors. Multivariable regression analyses were carried out at EU level. However, in order to investigate the consistency of analyses made at EU level, results from countries with prevalence below and above the

9 According to the survey protocol, all samples were to reach the laboratory within 24 hours of sampling. In exceptional situations (for example, long journeys, weekends and public holidays) that period could be extended to 80 hours.

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

EU[11] median prevalence were subjected to additional analyses. Moreover, because of the particular case of Hungary, which had an exceptionally high prevalence of 85.6%, it was decided to investigate the impact of its data on the EU level outcome. Therefore, the multivariable regression analyses were also run for the total dataset with Hungarian data excluded, and also for Hungary separately.

4.3.4.1. Analysis of multicollinearity among potentially associated factors

Data were further analysed for evidence of association among potentially associated factors, since they may correlate with each other, or one may completely explain the association of another (collinearity). The Variance Inflation Factor (VIF) was used as a formal method to detect correlation among risk factors (multicollinearity). This factor measures how much the variances of the estimated regression coefficients are inflated compared to when the predictor variables are not linearly related. Essentially, each potential risk factor is used as the outcome in a regression analysis (described in detail in Appendix A, section 3). A VIF value that equals 1 indicates that there is no correlation among risk factors, whereas VIF values greater than 1 indicate a correlation. VIF values exceeding 10 are interpreted as an indication of strong multicollinearity.

4.3.4.2. Statistical model

Given the use of a binary outcome variable (*Salmonella*-positive or -negative status of broiler carcasses) with only two, mutually exclusive values (which were coded as 1 when the survey test was positive and 0 otherwise), logistic regression was the model of choice. However, as previously performed in the prevalence estimation (Report Part A - EFSA, 2010a), certain data properties needed to be taken into account in the analysis. The data analysed originated from a complex survey design and the aspects described in the following section were considered.

4.3.4.2.1. Aspects of clustering and of weighting of results

The clustering of results could result from several factors. Broiler carcasses, which were the epidemiological units of the analysis, sampled at the same slaughterhouse would have been exposed to the same conditions and risk factors, including those on which no information was available in the current survey but that might have been associated with *Salmonella* contamination. The rearing and pre-harvest processes, including comparable managerial and hygiene practices of farming and transportation of broiler flocks, are likely to be more similar among broiler batches processed in the same slaughterhouse than among broiler batches processed in different slaughterhouses. Similarly, the contamination by *Salmonella* of broiler carcasses processed in the same slaughterhouse may also correlate because of common processing and hygiene conditions and potential cross-contamination. Therefore, the risk for *Salmonella*-positive samples collected at the same slaughterhouse may be more similar than for samples collected at different slaughterhouses and such observations cannot be considered as independent observations in statistical analysis. Consequently, correlation among outcomes in those carcasses slaughtered at the same slaughterhouse, which induces extra variation (heterogeneity) between slaughterhouses (clusters), was taken into account in the statistical analysis of the effects of risk factors by including, in the regression model, a slaughterhouse-specific effect (random intercept[12] parameter, which is a random variable representing the effect of factors shared by carcasses processed in the same slaughterhouse) for the outcome of interest (*Salmonella* carcass contamination). The assumption underlying this type of model is that each slaughterhouse, and consequently each carcass processed in that slaughterhouse, is characterised by a certain baseline level of risk of contamination, regardless of the exposure to factors considered in the survey. It is noteworthy that the interpretation of the regression coefficients (odds ratios - ORs) in this model is conditional on the slaughterhouse-specific effects and that they cannot be interpreted as describing population-averaged effects of factors. This means that the obtained ORs are to be interpreted relative to slaughterhouses having comparable risk factors. Possible country confounding effects were also

11 Two non-MSs, Norway and Switzerland, were included in the overall EU level dataset.

12 The "baseline risk of a slaughterhouse" corresponds to the slaughterhouse's random intercept, because, by definition, the intercept is the value of the outcome when all factors (predictors) in the model are at the baseline value.

taken into account in the analysis by including the factor 'country' as a fixed effect in the model. More precisely, the effect of country was considered by including a country-specific intercept. Thus, logistic mixed-effects models were fitted with the effect of the slaughterhouse included as a random effect and the effect of the country as a fixed effect. These mixed-effects models enabled investigating differences in the outcome (*Salmonella* carcass contamination) between slaughterhouses, within countries. More detailed explanations about how to take account of correlation among observations in the statistical analysis of the effects of risk factors can be found in EFSA's Report of the Task Force on Zoonoses Data Collection on the statistical analysis of temporal and spatial trends of zoonotic agents in animals and food (EFSA, 2009a).

Weights were applied to the results. This was because the sample size did not reflect a country's broiler population size resulting in unequal sampling probabilities (of batches/carcasses) in countries. Consequently, for the analyses of the effect of potential risk factors, weights were applied during the statistical analysis. The weight to account for the disproportionate sampling of slaughtered broilers within a country was calculated as the ratio of the number of slaughtered broilers during a year in a country and the number of broilers sampled in the same country.

More detailed explanations on analytical methods are given in Appendix A.

4.3.4.2.2. Model building for *Salmonella* contamination, at EU level

The full (initial) model investigating *Salmonella* contamination included all the main effects without any interaction terms (additive model). Next a weighted random effects model was fitted, where the cluster corresponded to the slaughterhouse. One by one the factors which were not significant were discarded (backward procedure), starting with the largest *P*-value based on the Type III test (Wald's test). Only those factors with *P*-values smaller than 0.05 were retained in the final model. The significance of the random effects was tested using the Wald test and a 50:50 mixture of Chi-square distributions with 0 and 1 degrees of freedom (Molenberghs and Verbeke, 2005).

With the aim of visualising the variability between slaughterhouses with respect to the random effect (random intercept), as estimated by the final model for *Salmonella* contamination of carcasses, a plot was produced that displayed per country for every surveyed slaughterhouse, the estimate of the slaughterhouse-specific effect (the random intercept), while adjusting for the country level fixed effect.

4.3.4.2.3. Analysis of the variance explained by the slaughterhouses

According to the outcome of the random effects models, the total variability could be split into two parts: one part explained by the investigated factors included in the model and a remaining unexplained part. The latter unexplained variance might be due to factors for which no data were gathered during the survey. However, even in the hypothetical case that all existing risk factors for *Salmonella* contamination results would have been included in the model, there could still be a certain amount of unexplained variance due to the fact that *Salmonella* is an infective agent, leading to the clustering of *Salmonella* contamination results within slaughterhouses. This unexplained variance was further investigated to quantify the proportion attributable to random effects (slaughterhouse-specific effects). Therefore, the intra-slaughterhouse or intra-cluster correlation coefficient (ICC) was estimated and was approximated as the ratio of the variance of the random effects and the sum of the variance of the random effects and the variance of the standard logistic density (Molenberghs and Verbeke, 2005). An ICC ranges between zero and one and corresponded respectively to scenarios of low (closer to zero) or high (closer to one) proportions of unexplained variance that was due to random effects (slaughterhouse-specific effects, between-slaughterhouse variability). In the latter case the *Salmonella* contamination results of broiler carcasses within a slaughterhouse are very much associated (alike). Caution is warranted while interpreting the ICC, because no conclusions can be made as regards the sources of the unexplained variance captured by the random intercept. This is because the proportion of unexplained variance due to random effects might be attributable either to uninvestigated slaughterhouse-specific effects or to the clustering of *Salmonella* contamination results.

Details on the calculations of the ICC in the context of the used random effects models are presented in Appendix A.

4.4. Analysis of *Salmonella* serovars distribution

The *Salmonella* serovars isolated on the broiler carcasses during this EU survey were previously reported in the Part A report.

Frequency distributions of isolated *Salmonella* serovars were analysed in detail, by country.

4.4.1. Spatial distribution of reported *Salmonella* serovars

Prevalence maps were produced displaying spatially country-specific prevalence for the serovars isolated by at least nine countries, i.e. *S.* Infantis, *S.* Agona, *S.* Hadar, *S.* Mbandaka, *S.* Enteritidis and *S.* Typhimurium.

4.4.2. Comparison between *Salmonella* serovar distributions in broiler carcasses, broiler flocks, other animal sources, feed and humans

The serovar distribution found on broilers carcasses was compared with the serovar distribution found in other animal sources, animal feed and humans. To this end, data were obtained from the BSs for laying hen flocks (EFSA, 2007b), broiler flocks (EFSA, 2007c), slaughter pigs (EFSA, 2008a), and turkey flocks (EFSA, 2008b). Human and feed data were obtained from the Community Summary Reports (CSRs) (EFSA, 2006; 2007d; 2009b; 2010c).

The descriptive analysis of the serovar data was performed in SAS Enterprise Guide 3.0 and Microsoft Excel. Tables and bar graphs were constructed using Microsoft Office Excel 2003. For the box plots, the software used was STATA/IC® 11.0 for Windows.

4.5. Simulation-based assessment of *Salmonella* process hygiene criteria in poultry meat

A simulation exercise was performed by EFSA's Assessment Methodology Unit to investigate the MS-specific probability of meeting the *Salmonella* microbiological process hygiene criteria in poultry meat as laid down by Regulation (EC) No 2073/2005 and its amendments, based on the prevalence of *Salmonella*-contaminated broiler carcasses. The outcome report is published as a stand-alone document (EFSA, 2011) and must be read as part of the present report part B in order to fully appreciate assumptions, uncertainties and data used in the modelling work. A simplified deterministic approach was opted for, based on the simulation of and the comparison between different scenarios. The simulation focussed on the carcass prevalence at slaughterhouse level and its corresponding probability of meeting the microbiological process hygiene criterion.

EFSA's Assessment Methodology Unit were asked to:

- build a model linking process hygiene criteria at production level and/or food safety criteria at retail to the baseline prevalence estimates at production or at retail in foodstuffs. The resulting models should allow for:
    - testing if MSs meet the microbiological criteria in place, and
    - investigating the impact of alternative microbiological criteria via simulations.
- validate the statistical models (model fit, model comparison);
- write short but comprehensive technical guidelines on how to use the model in the upcoming analysis. This should include a clear list of assumptions to be made and/or checked as well a list of input and output data; AND

- implement a simulation-based example to illustrate the use of such model.

5. Results

5.1. Factors associated with *Salmonella*-contaminated broiler carcasses

5.1.1. Descriptive analysis of factors potentially associated with *Salmonella*-contaminated broiler carcasses

Univariable description and bivariable association of factors potentially associated with *Salmonella*-contaminated broiler carcasses are presented in full in Appendix B (Tables 8 to 25 and Figures 9 to 25). The most interesting results are displayed hereafter.

5.1.1.1. Time (hour) of sampling during the day

Figure 1 depicts the prevalence for *Salmonella*-contaminated broiler carcasses according to the time (hour) of sampling during the day. The EU level prevalence of contaminated carcasses seems to increase during the afternoon.

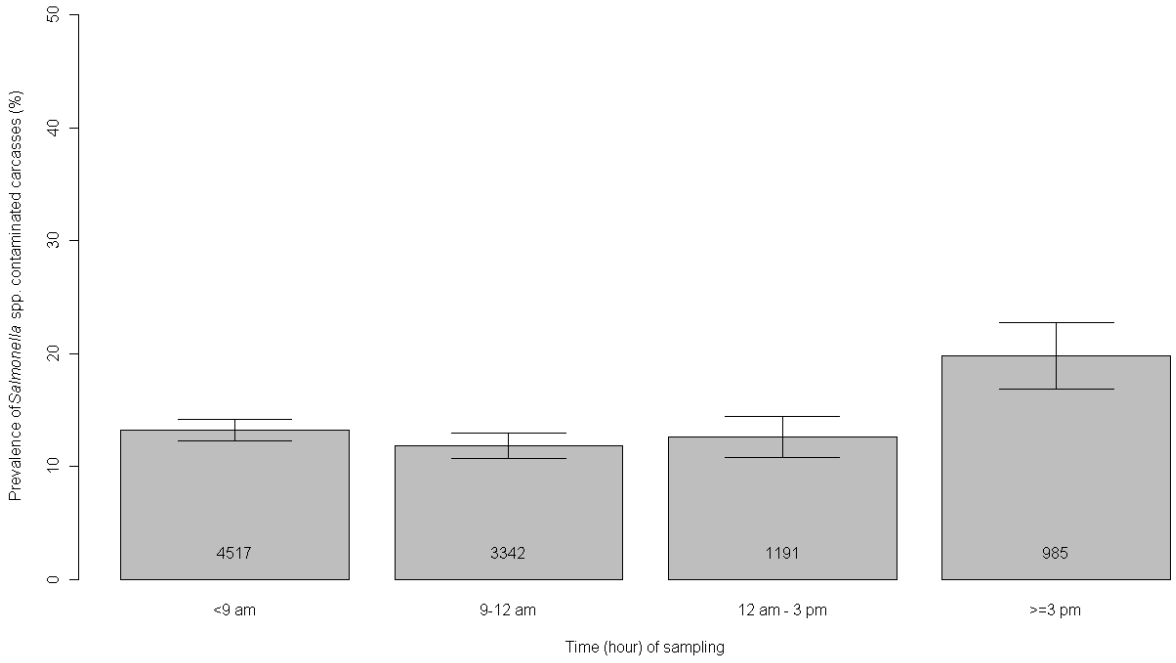

Figure 1: Prevalence of *Salmonella*-contaminated broiler carcasses by time (hour) of sampling in the EU [(a)], 2008

Note: The numbers appearing within the shaded areas of the bars indicate the total number of sampled broiler carcasses for each category.

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

5.1.1.2. Slaughter capacity of slaughterhouse

Figure 2 displays the barplot of the EU level prevalence of *Salmonella*-contaminated broiler carcasses according to slaughterhouse capacity showing that prevalence increases as the capacity of the slaughterhouse increases, up to a capacity of 5,000,000; thereafter, prevalence tended to decrease.

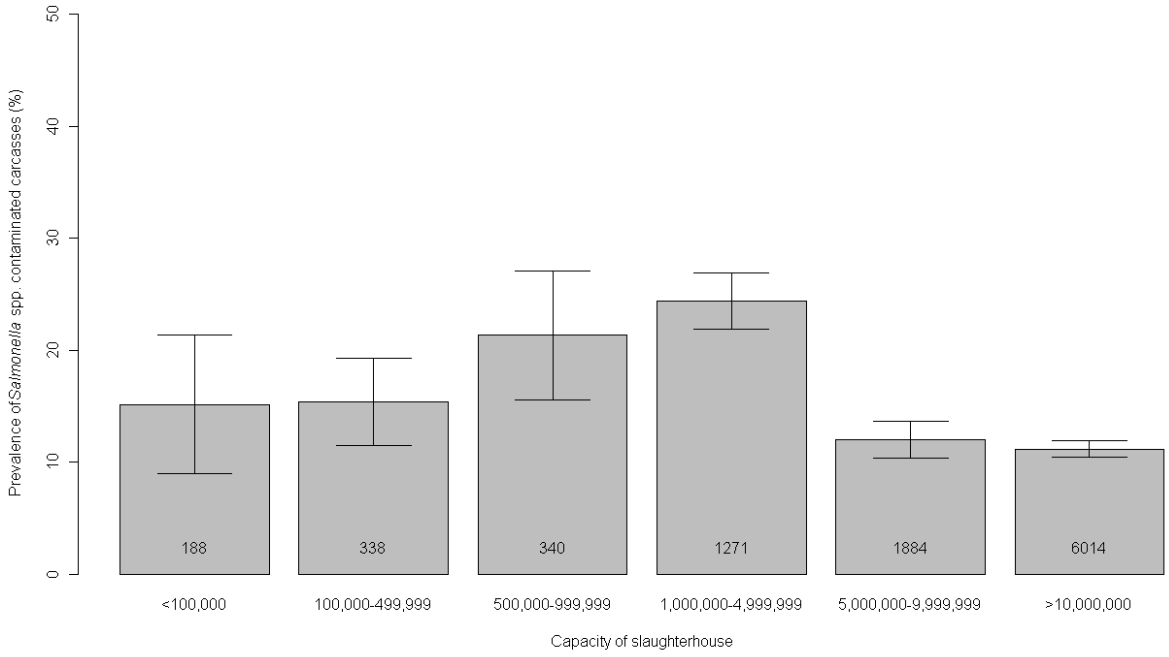

Figure 2: Prevalence of *Salmonella*-contaminated broiler carcasses by slaughterhouse capacity in the EU [(a)], 2008

Note: The numbers appearing within the shaded areas of the bars indicate the total number of sampled broiler carcasses for each category.

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis. Analysis of multicollinearity among potentially associated factors.

The VIF values calculated for the multicollinearity analysis among the factors associated with *Salmonella*-contaminated broiler carcasses are presented in Appendix C, Table 26, namely flock production type, previous thinning in the flock, age of broilers, quarter of sampling, time (hour) of sampling, hours between sampling and testing, capacity of slaughterhouse, type of chilling, and *Campylobacter* contamination result on the broiler carcass. This analysis showed that multicollinearity was not important for the full model since all the VIF values were close to 1.

5.1.2. Identification of factors potentially associated with *Salmonella*-contaminated broiler carcasses

A full random effects model was fitted including all the available factors: country, flock production type, previous thinning in the flock, age of broilers, quarter of sampling, time (hour) of sampling, hours between sampling and testing, capacity of slaughterhouse, type of chilling, and *Campylobacter* contamination result on the broiler carcass.

The factors that were discarded based on the backward selection procedure were, consecutively: flock production type, quarter of sampling, hours between sampling and testing, type of chilling, *Campylobacter* result on broiler carcasses, age of broilers, and previous thinning in the flock. The following risk factors for *Salmonella*-contaminated broiler carcasses were retained in the final logistic mixed effects model:

- time of sampling during the day;
- capacity of the slaughterhouse.

The OR estimates for the factors in the final model at EU level are presented in Table 3.

Table 3: Final logistic mixed effects model [(a)] for factors associated with *Salmonella*-contaminated broiler carcasses, in the EU [(b)], 2008

Factor	Level	Odds ratio [(c)]	95 % CI		*P*-value
Time (hour) of sampling	9 - < 12am	0.972	0.708	1.335	0.0003
Reference category: < *9am*	12am - < 3pm	1.674	1.156	2.423	
	≥ 3pm	2.680	1.646	4.365	
Capacity of the slaughterhouse		1.951	1.313	2.900	0.0009

(a): Estimates and standard errors were assessed using a mixed effects model with a random effect on the intercept to take account of slaughterhouse effects and with the factor 'country' as a fixed effect.
(b): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.
(c): All ORs were adjusted for country effect.

In Table 3, an OR >1 indicates that exposure to the factor increases the risk of *Salmonella* contamination, whereas an OR <1 indicates a negative association between the factor and contamination. An OR equal to 1 indicates no effect of the factor on *Salmonella* contamination. Consequently, if the 95% CI of the OR does not comprise 1, meaning that both the lower and the upper limits are either greater, or less than 1, it can be concluded that the association with a potential factor and *Salmonella* is statistically significant ($P < 0.05$).

The final model included country-specific effects (not shown) and ORs are, therefore, adjusted for countries. According to the analyses, the risk of *Salmonella* contamination of carcasses increased as the capacity of the slaughterhouse increased, because the odds of having a positive *Salmonella* result on broiler carcasses is higher for slaughterhouses with a larger capacity. However, as explained in the Materials and Methods section for this particular risk factor, interpretation of this OR should focus mainly on the direction of the effect, without giving too much emphasis (interpretation) on the size of the effect. Secondly, the odds of having a positive *Salmonella* result increased also when the carcass was processed later during the day. In particular, the ORs were significantly different when the samples were collected in the afternoon (≥ 12 am), compared to before 9 am.

The variance of the random effects (effect of slaughterhouses) in the final regression model was significantly different from zero (*P*-value <0.001, Appendix E, Table 28). This indicated that the baseline risk of *Salmonella* carcass contamination varied between the slaughterhouses, even when accounting for other factors i.e. the time of processing the carcass during the day, the capacity of the slaughterhouse and the factor 'country'. Consequently, within countries, there were slaughterhouses with an overall higher prevalence and slaughterhouses with an overall lower prevalence of *Salmonella*-contaminated carcasses. The proportion of variance (in *Salmonella* contamination results) that remained unexplained by the investigated factors and that was due to between-slaughterhouse variability was 46%.

Figure 3 visualises, for every country, the variability between slaughterhouses with respect to the random effect (random intercept) estimated by the final model for *Salmonella* contamination of carcasses. The plot is ranked by the MS-specific prevalence of *Salmonella*-contaminated broiler carcasses in the participating countries. The dashed line represents the value 0 for these random effects. Slaughterhouses with their estimated random effect below this line have a smaller intercept and thus a lower prevalence of *Salmonella*-contaminated carcasses; slaughterhouses above have a higher prevalence of *Salmonella*-contaminated carcasses. It is emphasised that Figure 3 displays estimated random effects specific for each surveyed slaughterhouse. These are not adjusted for the country level fixed effect. Consequently, no exact prevalence values can be inferred from this plot.

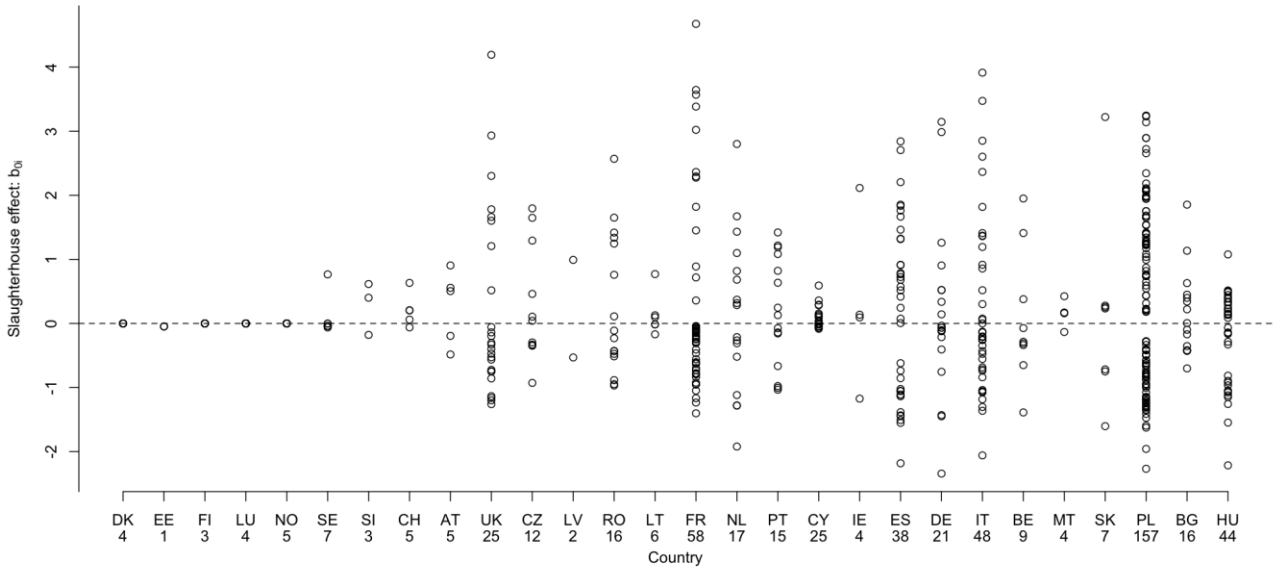

Figure 3: Plot of slaughterhouse random effects ranked by the prevalence of *Salmonella*-contaminated broiler carcasses, by country, in the EU [(a)], 2008

Note: The dashed line stands for the value 0 for these random effects. The numbers indicated below the country abbreviation indicate the number of sampled slaughterhouses. The slaughterhouse random effect for Estonia (EE) should be interpreted with caution, as this country had only one slaughterhouse sampled.

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

An additional complementary analysis following the above modelling approach was made to compare the full model results for *Salmonella*-contaminated carcasses between two groups of countries having higher and lower batch prevalence. The arbitrary cut-off was chosen as the EU[13] median prevalence dividing the countries into two groups with a prevalence of *Salmonella*-contaminated broiler carcasses below and above 6.4%, respectively. The results are displayed in Appendix D, Table 27.

For the group of countries with prevalence below 6.4%, the only factor associated with *Salmonella*-contaminated carcasses that was statistically significant in the full mixed effects model was the type of chilling used for the carcasses. The risk of *Salmonella* contamination of carcasses was estimated lower if broiler carcasses were chilled by a mixed-chilling method. However, since these results are based on a full – not a final – model, the observed effect of the type of chilling used is only indicative. The variance of the random effects (effect of slaughterhouses) in the regression model was not significantly different from zero. This indicated that the baseline risk of *Salmonella* carcass contamination did not vary between the slaughterhouses, for this country group.

[13] Two non-MSs, Norway and Switzerland, were included in the overall EU level dataset.

For the group of countries with prevalence above 6.4%, the following potential factors associated with *Salmonella*-contaminated broiler carcasses were statistically significant in the full logistic mixed effects model:

- the time (hour) of sampling during the day; and
- the capacity of the slaughterhouse.

Although these results are only indicative because they are based on a full – not a final – model, they were consistent with the results obtained by the final random effects model that was fitted using the overall EU level dataset and all the available factors. Also, the variance of the random effects (effect of slaughterhouses) was significantly different from zero. This indicated that the baseline risk of *Salmonella* carcass contamination varied between the slaughterhouses for this country group. In this subgroup, the proportion of variance (in *Salmonella* contamination results) that remained unexplained by the investigated factors and that might have been due to between-slaughterhouse variability was 49%. Hence, slaughterhouse-specific effects impacting on the risk of contamination of broiler carcasses were likely to be much stronger (more important) compared to the effects in the lower prevalence country group.

An additional complementary analysis, following the above modelling approach, was made to compare the full model results for *Salmonella*-contaminated broiler carcasses between the total EU dataset and the dataset without the Hungarian data. The results of the analyses covered by the latter dataset were consistent with the results obtained from the total EU level dataset presented previously. The variance of the random effects (effect of slaughterhouses) in the analyses covered by the dataset without the Hungarian data was also significantly different from zero.

This means that the Hungarian data (exceptionally high *Salmonella* contamination) did not have an important effect on the analyses and results at EU level.

5.2. Analysis of the *Salmonella* serovars distribution

5.2.1. Frequency distribution of *Salmonella* serovars on *Salmonella*-contaminated broiler carcasses

The *Salmonella* serovars isolated from the broiler carcasses were previously reported in the Part A report. A total of 9,249 carcasses from the 26 MSs, 390 from Switzerland and 396 from Norway were sampled, adding up to 10,035 tested units. This resulted in 1,225 (1,215 from the EU) positive samples and 1,261 isolates, since 31 samples from France, Germany and Hungary had two or in some cases, three isolates from the same sample.

In the EU, 13.1% of carcasses were positive for *Salmonella*, while the overall positivity was lower (12.2%), due to the fact that Norway had no positive samples. The highest positivity was observed in Hungary (85.7%), with a large difference to the second and third highest, Bulgaria (26.9%) and Poland (25.5%). Denmark, Estonia, Finland, Luxembourg and Norway had no positive samples. The number of samples submitted, number and percentage of positives and number of serovars found in each country are shown in Table 4. Fifty-six serovars were reported by 22 MSs and Switzerland in this survey. In countries with positive samples, the number of isolated serovars varied from one in Ireland, Latvia and Sweden to 16 in Bulgaria.

Table 4: Number of submitted and positive samples, positivity percentage and number of serovars reported on broiler carcasses in the EU [(a)], 2008.

Country	Samples (n)			No of different serovars reported [(b)]
	Tested	Positive	% Positive	
Austria	408	10	2.5	6
Belgium	380	77	20.3	12
Bulgaria	316	85	26.9	16
Cyprus	357	38	10.7	8
Czech Republic	422	23	5.5	7
Denmark	396	0	0	0
Estonia	102	0	0	0
Finland	369	0	0	0
France	422	32	7.6	13
Hungary	321	275	85.7	5
Germany	432	76	17.6	14
Ireland	394	39	9.9	1
Italy	393	66	16.8	13
Latvia	122	6	4.9	1
Lithuania	374	26	7.0	8
Luxembourg	13	0	0	0
Malta	367	77	21.0	7
Netherlands	429	43	10.0	9
Poland	419	107	25.5	11
Portugal	421	47	11.2	4
Romania	357	17	4.8	6
Slovakia	422	91	21.6	10
Slovenia	413	7	1.7	3
Spain	389	58	14.9	14
Sweden	410	1	0.2	1
United Kingdom	401	14	3.5	9
EU Total	**9,249**	**1,215**	**13.1**	**56**
Switzerland	390	10	2.6	5
Norway	396	0	0	0
Total	**10,035**	**1,225**	**12.2**	**56**

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.
(b): Untypeable isolates were not considered.

The leading isolated serovar was *S.* Infantis (29.2% of positive carcasses). However, this was explained, to a large extent, by the contribution from a single MS, Hungary, which accounted for 75.1% of all *S.* Infantis isolates. *S.* Enteritidis was the second most frequently isolated serovar, found in 13.6% positive carcasses. *S.* Kentucky was followed by *S.* Typhimurium with 6.2% and 4.4% positive carcasses, respectively, although *S.* Typhimurium was more spread across Europe. In contrast, *S.* Kentucky and *S.* Paratyphi B var. Java were concentrated in a few countries, but their presence in those countries was generally high. *S.* Hadar, *S.* Agona and *S.* Mbandaka were observed in 3.8%, 3.0%, and 2.4% of samples, respectively. Together with *S.* Enteritidis and *S.* Typhimurium, these serovars were highlighted in the subsequent analyses due to their wide-spread distribution in broiler carcasses across the EU and because all, except *S.* Mbandaka, feature on the human top 10 serovars

responsible for human *Salmonella* infections in the EU (Table 6, Table 30). The top 20 serovars isolated from broiler carcasses in the survey are presented in Table 30 (Appendix F), ordered by percentage among positive isolates. No analyses on the distribution of *S.* Enteritidis or *S.* Typhimurium phage types were performed, due to lack of reported data.

No specific serovar was predominant in all countries. However, *S.* Enteritidis was present in 14 countries, being the dominant serovar in five MSs: Latvia, Poland, Portugal, Slovakia and Spain. From the only positive sample in Sweden, *S.* Agona was isolated, and it was also the main serovar in Lithuania and in the Czech Republic. Another dominant serovar at country level was *S.* Kentucky, which accounted for 100% of Irish isolates (n=39), and was also relatively frequent in Malta, Slovakia and in the United Kingdom. *S.* Infantis is a particular case, as it accounted for 97.8% of Hungarian *Salmonella*-contaminated carcasses (n=275), but was not confined to this country, being present in 14 MSs and Switzerland. This makes it the most widely distributed serovar in the survey. *S.* Typhimurium was observed in nine MSs and Switzerland, and *S.* Mbandaka, isolated in 10 MSs, appeared to be present to a certain extent in Poland and Portugal. *S.* Hadar was detected in nine MSs, and was particularly frequent in Italy and Cyprus.

The number of countries where the top 20 serovars were found is presented in Table 31 (Appendix F). The relative proportion of *S.* Infantis, *S.* Agona, *S.* Hadar, *S.* Mbandaka, *S.* Enteritidis and *S.* Typhimurium in countries with positive samples is displayed in Figure 4.

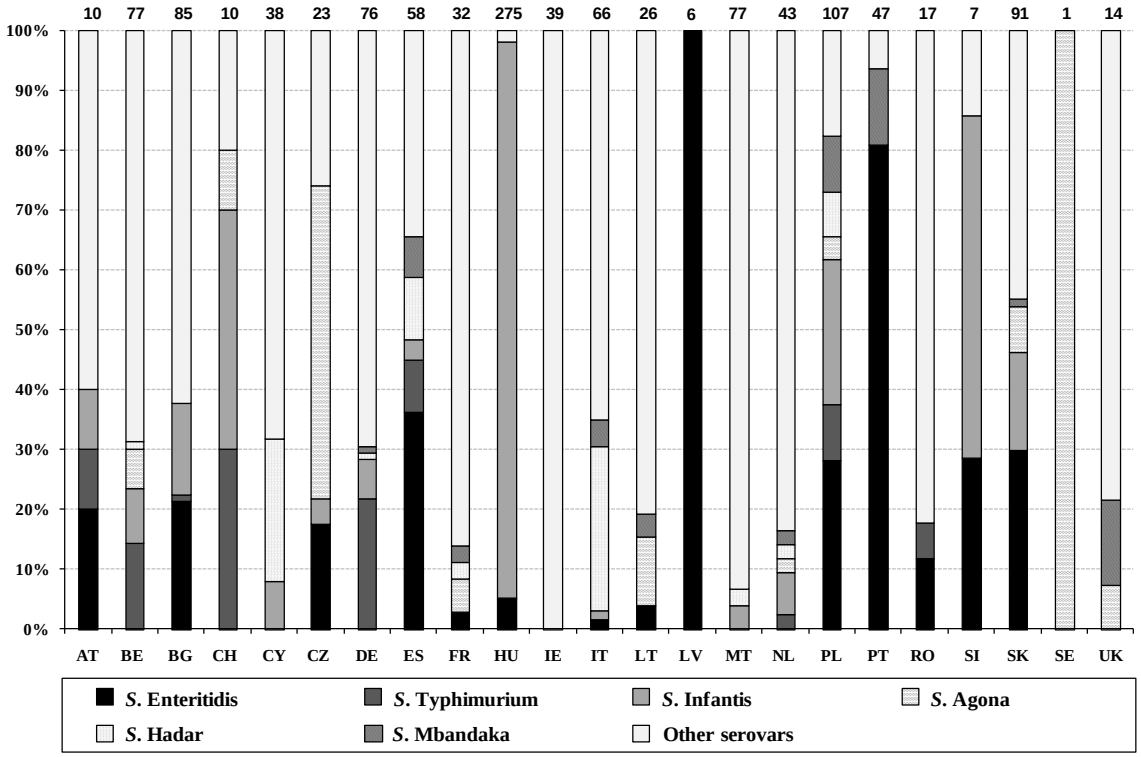

Figure 4: Relative frequency distribution (%) of *S.* Infantis, *S.* Agona, *S.* Hadar, *S.* Mbandaka, *S.* Enteritidis and *S.* Typhimurium on broiler carcasses in EU[(a)] MSs

Note: The numbers on top of the bars show the total positive samples, corresponding to 100% for each bar.
(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

5.2.2. Spatial distribution of *Salmonella* serovars on *Salmonella*-contaminated broiler carcasses

EU maps with serovar-specific prevalence estimates for *S.* Infantis, *S.* Agona, *S.* Hadar, *S.* Mbandaka, *S.* Enteritidis and *S.* Typhimurium in countries participating in the broiler carcass BS 2008 can be found in Appendix G, Figures 26 to 31.

5.2.3. Comparison between *Salmonella* serovar distributions in broiler carcasses, broiler flocks, other animal sources, feed and humans

The majority of the serovars isolated from broiler carcasses has also been isolated previously from flocks with broilers and flocks with laying hens, suggesting the existence of common sources of infection. *S.* Infantis and *S.* Enteritidis were the two most frequently isolated serovars in broilers (flocks and carcasses) and flocks with laying hens. The majority of the most commonly detected *Salmonella* serovars among turkey flocks were also listed among the top serovars in broiler flocks and broiler carcasses. An exception seems to be *S.* Saintpaul occurring frequently in turkeys (EFSA, 2008b), but not in broilers. Similar conclusions can be drawn when comparing with the frequency distribution of *Salmonella* serovars isolated from slaughter pigs (lymph nodes and carcass swabs), where there also exists some overlap between the most common serovars, although the ranking of the serovars in order of frequency differs between the two sources. Feed is a plausible source of a part of these infections and, as can be seen in Table 5, 17 of the top 20 serovars detected on the broiler carcasses have also been reported in feed. It is underlined that the data in Table 5 are based on what is reported by the MSs in the BS reports or for feed in the CSR. The fact that a serovar has not been reported does not necessarily means that it does not exist in the source in question.

Table 5: Frequency of *Salmonella* serovars reported in the Community Summary Report on feed [(a)] and in the baseline surveys on broiler carcasses, broiler flocks, turkey flocks, laying hen holdings and slaughter pigs [(b)]

Salmonella serovar	Broiler carcasses with serovar	Detected in feed (unspecified poultry feed, or oil seed and fruit)	Flocks with broilers (in top 20 serovars)	Flocks with laying hens (in top 20 serovars)	Flocks with fattening turkeys (in top 20 serovars)	Slaughter pigs (lymph nodes with serovar)	Slaughter pigs (carcass swabs with serovar)
S. Infantis	358	Yes	295	171	72	49	13
S. Enteritidis	167	Yes	538	899	55	126	5
S. Kentucky	76	Yes	44	12	-	-	1
S. Typhimurium	66	Yes	65	123	86	1,040	191
S. Bredeney	53	Yes	10	26	186	51	8
S. Virchow	50	Yes	30	41	11	7	1
S. Hadar	47	Yes	59	53	152	8	1
S. Paratyphi B var. Java	46	Yes	-	-	-	2	-
S. Agona	37	Yes	16	38	31	28	4
S. Indiana	36	Yes	19	11	32	-	-
S. Montevideo	33	Yes	31	27	13	19	-
S. Mbandaka	30	Yes	114	101	9	7	-
S. Blockley	22	No	29	4	40	2	-
S. 4,12:d:-	22	No	-	-	-	-	-
S. Thompson	21	Yes	-	-	-	9	-
S. 4,[5],12:i:- [(c)]	15	No	-	-	-	104	4
S. Livingstone	12	Yes	39	50	-	9	4
S. 6,7:-:-	11	Yes	-	-	-	-	-
S. Ohio	11	Yes	19	35	-	7	1
S. Derby	10	Yes	13	14	123	380	94

(a): EFSA Community Summary Report 2007 (EFSA, 2009b).
(b): EFSA baseline surveys on broiler carcasses (EFSA , 2010a), broiler flocks (EFSA, 2007c), turkey flocks (EFSA, 2008b), laying hen holdings (EFSA, 2007b) and slaughter pigs (EFSA, 2008a).
(c): According to EFSA's BIOHAZ panel scientific opinion on monitoring and assessment of the public health risk of "*Salmonella* Typhimurium-like" strains (EFSA, 2010d), this *Salmonella* antigenic formula is recommended to be reported as 'monophasic *Salmonella* Typhimurium'. However, to ensure consistency with the previously published Part A report (EFSA, 2010a), the *Salmonella* antigenic formula is kept here.

A closer look at the serovar distribution in broiler carcasses and broiler flocks, shows that in general, serovars tend to be the same and occur in similar proportions from both sources, with small differences. Exceptions can be observed in two MSs. In Cyprus, *S.* Enteritidis and *S.* Agona were the main serovars in flocks, whereas in carcasses, *S.* Hadar and *S.* Infantis dominate. In Ireland, a high proportion of *S.* Mbandaka was observed in flocks but, as previously mentioned, all 39 isolates from the Irish carcass survey were *S.* Kentucky (shown as "others" in Figure 5).

Figure 5 presents the relative distribution of *S.* Infantis, *S.* Agona, *S.* Hadar, *S.* Mbandaka, *S.* Enteritidis and *S.* Typhimurium, according to data from the BS on broiler flocks 2005-2006 (EFSA, 2007c), in countries which had positive samples for both studies. The fact that the surveys in broiler flocks and carcasses were conducted two years apart, indicate that the majority of the frequently encountered serovars are well-established in broiler production in most countries.

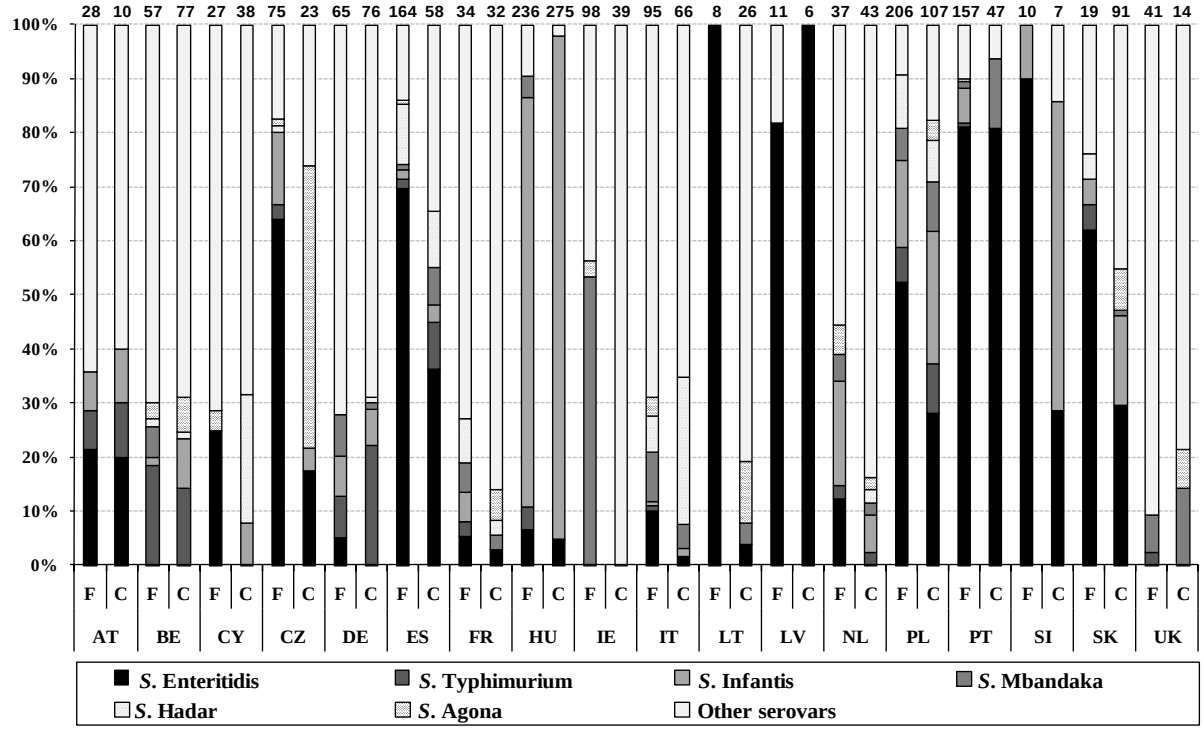

Figure 5: Relative distribution of *S.* Infantis, *S.* Agona, *S.* Hadar, *S.* Mbandaka, *S.* Enteritidis and *S.* Typhimurium in broiler flocks (F) and broiler carcasses (C) in 18 MSs

Note: The numbers on top of the bars show the total positive samples, corresponding to 100% for each bar.

When taking estimated prevalence into consideration, Figure 6 shows the distribution of values for *S.* Infantis, *S.* Hadar, *S.* Mbandaka, *S.* Enteritidis, *S.* Typhimurium and *S.* Typhimurium or *S.* Enteritidis in broiler flocks (EFSA, 2007c) and broiler carcasses. The prevalence for *S.* Agona could not be estimated in a reliable way in broiler flocks, therefore it was not included in the figure. The box plot shows that serovar-specific prevalence tended to concentrate around the same values for both levels, which is shown by the similar size and relative position of the boxes in the graph. However, carcasses seemed to have a wider range of prevalence results directed towards more extreme high values for *S.* Enteritidis, which is shown by the median line and the black dots above the box area. For *S.* Infantis, extreme values were observed for both levels, but was highest at flock level.

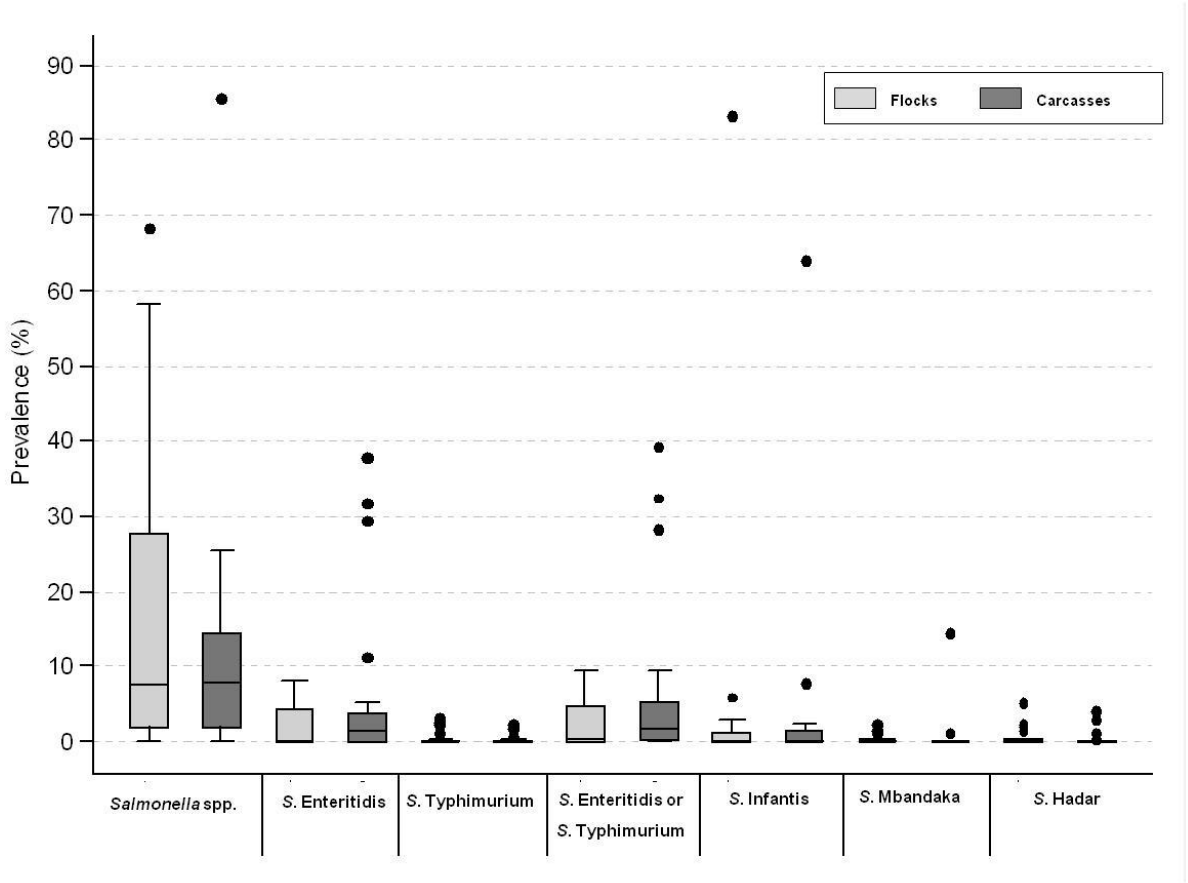

Figure 6: Prevalence distribution of *S.* Infantis, *S.* Hadar, *S.* Mbandaka, *S.* Enteritidis, *S.* Typhimurium and *S.* Typhimurium or *S.* Enteritidis in broiler flocks and broiler carcasses

Note: Bulgaria, Luxembourg, Malta, Romania and Switzerland did not have data from both studies and their results were not included in this graph.

Serovar information on humans was not available at MS level, so the comparison was made using EU totals. Table 6 shows the most important *Salmonella* serovars reported in humans in the EU from 2005 to 2008 (EFSA, 2006; 2007d; 2009b; 2010c). The reported human data represent aggregated data meaning that serovars reported individually in one year may be reported in the group "Other" for other years.

Table 6: Top 10 *Salmonella* serovars reported in humans in the EU, Community Summary Reports, 2005-2008 [(a)]

Serovar	Year							
	2005 (N=23 MSs + 2)		2006 (N=24 MSs + 4)		2007 (N=26 MSs + 3)		2008 (N=26 MSs + 3)	
	N	%	N	%	N	%	N	%
S. Enteritidis	86,536	53.7	90,362	71.0	81,472	64.5	70,091	58.0
S. Typhimurium	15,058	9.3	18,685	14.7	20,781	16.5	26,423	21.9
S. Infantis	1,354	0.8	1,246	1.0	1,310	1.0	1,317	1.1
S. Bovis morbificans	621	0.4	-	-	-	-	501	0.4
S. Hadar	577	0.4	713	0.6	479	0.4	-	-
S. Virchow	535	0.3	1,056	0.8	1,068	0.8	860	0.7
S. Derby	259	0.2	477	0.4	469	0.4	624	0.5
S. Newport	245	0.2	730	0.6	733	0.6	787	0.7
S. Stanley	-	-	522	0.4	589	0.5	529	0.4
S. Agona	-	-	367	0.3	387	0.3	636	0.5
S. Anatum	179	0.1	-	-	-	-	-	-
S. Goldcoast	173	0.1	-	-	-	-	-	-
S. Kentucky	-	-	357	0.3	431	0.3	497	0.4
Other	55,619	34.5	12,790	10.0	18,562	14.7	18,495	15.3
Total	**161,156**		**127,305**		**126,281**		**120,760**	
Unknown	56,619		17,359		9,814		6,636	

(a): EFSA Community Summary Reports, 2005-2008 (EFSA, 2006; 2007c; 2009b; 2010c).

Figure 7 presents the relative distribution of the selected serovars in humans (EFSA, 2010c), broiler flocks (EFSA, 2007c) and broiler carcasses in 2008. Although proportions are slightly different in the bars, the predominant serovars were the same, namely *S.* Enteritidis, *S.* Typhimurium and *S.* Infantis. *S.* Hadar, which was considered an important serovar in broilers, was among the most important in humans up to 2007, but was not present in the human top 10 *Salmonella* serovar list in 2008. When preparing Figure 7, it was considered that most isolates of *S.* Infantis in broilers came from Hungary, which could unbalance the true relative distribution of serovars in the EU. However, removing these isolates would only increase the relative percentage of *S.* Enteritidis (the second most common serovar) from 13.6% to 18.5%, and the other serovars would suffer even smaller changes. It was, therefore, decided to keep *S.* Infantis in the broiler carcass bar. No serovar-specific data on *S.* Hadar and *S.* Mbandaka in humans were available in 2008.

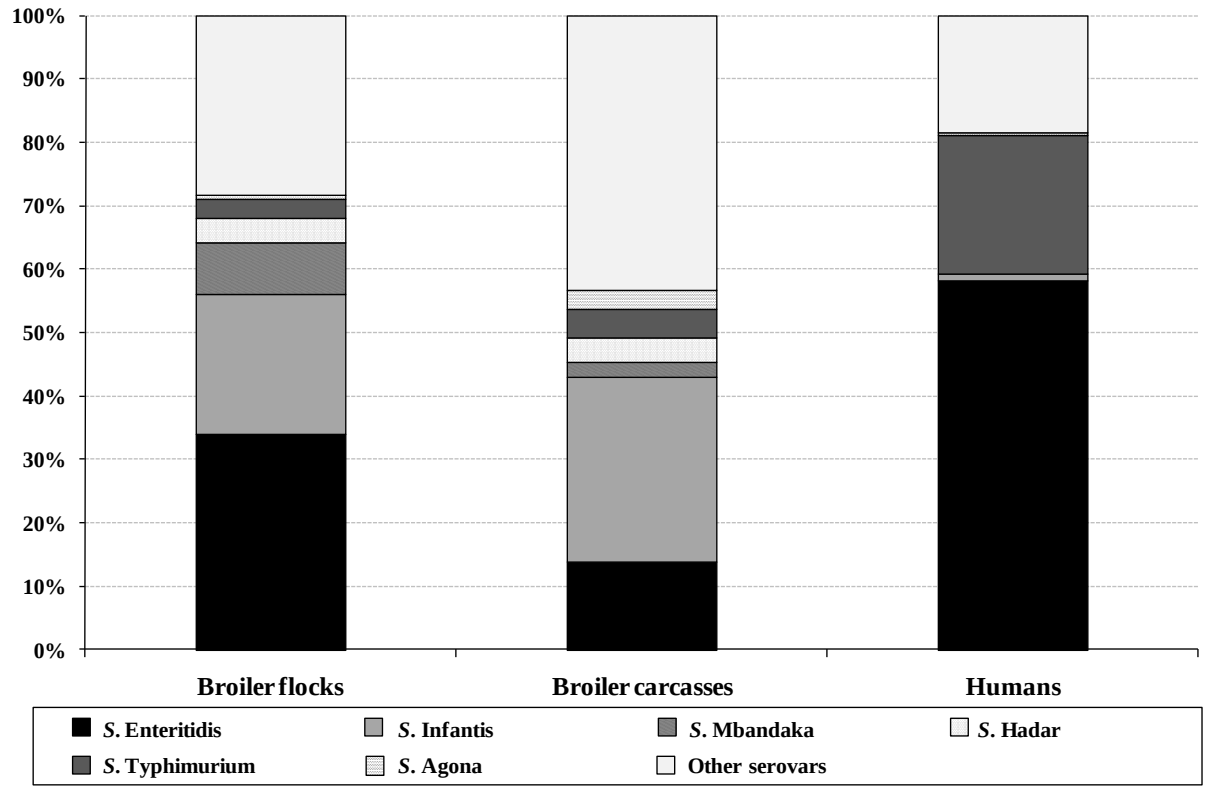

Figure 7: Comparison of the serovar distribution in humans (2008 data), broiler flocks (2006 data) and carcasses (2008 data) in the EU

5.3. Comparison between the *Salmonella* spp. prevalence estimates in broiler meat assessed by regular monitoring in 2008 and in the baseline survey in 2008

Figure 8 descriptively compares the *Salmonella* prevalence deriving from the 2008 routine monitoring of broiler meat reported by the seven MSs which reported this type of data in 2008 (EFSA, 2010c) and the BS prevalence. For this MS group, it seemed that for most MSs the prevalence for *Salmonella*-contaminated carcasses in MS in the BS was comparable to the prevalence reported by the MS for broiler meat for the regular monitoring results from 2008.

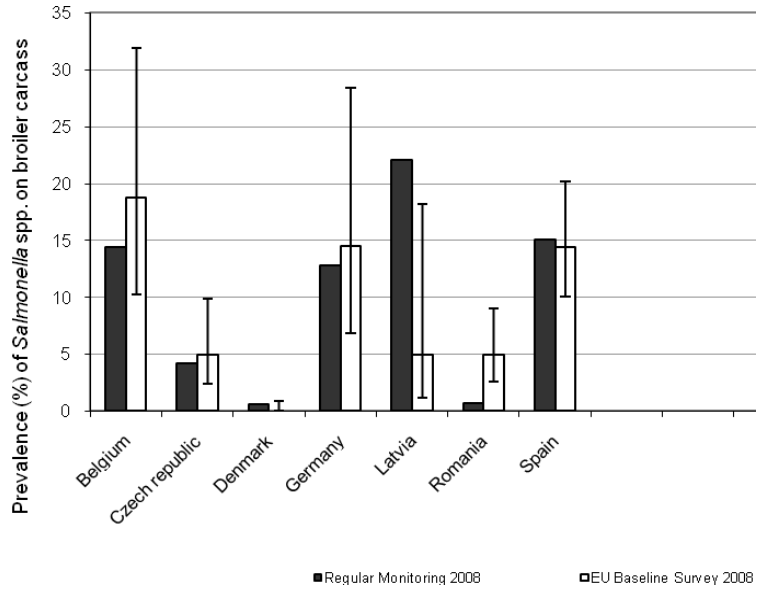

Figure 8: Comparison between the *Salmonella* spp. prevalence [(a)] estimates in broiler meat assessed by regular monitoring in 2008 and in the baseline survey in 2008 for seven MSs

(a): Vertical bars represent 95% CIs.

5.4. Simulation-based assessment of *Salmonella* process hygiene criteria in poultry meat

The MS-specific probability of meeting the *Salmonella* microbiological process hygiene criteria in poultry meat as laid down by Regulation (EC) No 2073/2005 and its amendments, based on the prevalence of *Salmonella*-contaminated broiler carcasses and their 95% CIs, is provided in Table 7. These outcomes assumed diagnostic sensitivities and specificities of respectively 90% and 100% for the baseline survey test as well as for the test of pooled samples of neck skin (sets of 50 pooled neck skin samples, consisting each of three individual neck skins) conducted according to Regulation (EC) No 2073/2005, and a constant average prevalence of *Salmonella*-contaminated broiler carcasses over time. Table 7 shows that for 12 countries the difference between the upper and the lower limit of the estimated probability of meeting the *Salmonella* process hygiene criteria was less than 3, which is very precise. In this MS-group, according to the simulation results, Denmark, Finland, Sweden and Norway and Switzerland highly likely meet the *Salmonella* microbiological process hygiene criteria in poultry meat, whereas the other countries of this MS-group would most likely not. For a second group of MSs the difference between the upper and the lower limit of the estimated probability was between 10 and 35, and Estonia and Slovenia would likely meet the criteria. For the remainder of the MSs, the difference between upper and lower limit of the estimated probability of meeting the *Salmonella* process hygiene criteria was more than 50, indicating that the outcomes of the simulation exercise were too uncertain. More details regarding the assumptions, uncertainties and data used in the simulation can be found in the scientific report on the simulation-based assessment of microbiological criteria on *Salmonella* in poultry meat (EFSA, 2011).

Table 7: Summary of the results from the simulation estimating the probability of meeting the microbiological process hygiene criteria in poultry meat per Member State, according to the prevalence from the baseline survey and following the microbiological process hygiene criteria in force. Sensitivity and specificity are respectively 90% and 100% for both tests

Country	BS Prev.[(a)]	95% Ll[(b)]	95% Ul[(c)]	True carcass prev. [(d)]	True carcass prev., Ll[(e)]	True carcass prev., Ul[(f)]	App. Pooled prev. [(g)]	App. Pooled prev., Ll[(h)]	App. Pooled prev., Ul[(i)]	Prob.[(j)]	Prob., Ll[(k)]	Prob., Ul[(l)]	Difference between Prob., Ul[(l)] and Prob., Ll[(k)]
Austria	**2.7**	1.3	5.5	**3**	1.4	6.1	**7.9**	3.8	15.5	**96.3**	47.2	100	52.8
Belgium	**18.7**	10.2	31.9	**20.8**	11.3	35.4	**45.3**	27.3	65.8	**0**	0	2	2.0
Bulgaria	**26.6**	20.1	34.3	**29.6**	22.3	38.1	**58.5**	47.8	68.7	**0**	0	0	0
Cyprus	**10.5**	7.5	14.6	**11.7**	8.3	16.2	**28**	20.7	37.1	**1.5**	0	15.3	15.3
Czech Republic	**4.9**	2.4	9.9	**5.4**	2.7	11	**13.9**	7	26.6	**59.8**	2.6	98.1	95.5
Denmark	**0**	0	0.9	**0**	0	1	**0**	0	2.7	**100**	100	100	0
Estonia	**0**	0	3.6	**0**	0	4	**0**	0	10.4	**100**	85.7	100	14.3
Finland	0	0	1	0	0	1.1	0	0	3	100	100	100	0
France	**7.4**	3.8	13.7	**8.2**	4.2	15.2	**20.4**	10.9	35.2	**17.2**	0.1	82.7	82.6
Germany	**14.5**	6.8	28.4	**16.1**	7.6	31.6	**36.9**	18.9	61.1	**0**	0	24.9	24.9
Hungary	**85.6**	79.5	90.1	**95.1**	88.3	100.1	**90**	89.9	90	**0**	0	0	0
Ireland	**11.2**	3.4	31.4	**12.4**	3.8	34.9	**29.6**	9.8	65.2	**0.9**	0	88.6	88.6
Italy	**17.4**	12.1	24.3	**19.3**	13.4	27	**42.8**	31.6	55	**0**	0	0.3	0.3
Latvia	**4.9**	1.2	18.2	**5.4**	1.3	20.2	**13.9**	3.6	44.3	**60.3**	0	100	100
Lithuania	**5.4**	2.2	12.4	**6**	2.4	13.8	**15.2**	6.4	32.3	**50.7**	0.3	98.7	98.4
Luxembourg	**0**	0	24.7	**0**	0	27.4	**0**	0	55.6	**100**	0	100	100
Malta	**19.3**	12.2	29.2	**21.4**	13.6	32.4	**46.4**	31.9	62.3	**0**	0	0.3	0.3
Netherlands	**10.1**	6.2	16.1	**11.2**	6.9	17.9	**27**	17.3	40.2	**2.4**	0	34.6	34.6
Poland	**25.4**	20.9	30.5	**28.2**	23.2	33.9	**56.7**	49.3	64	**0**	0	0	0
Portugal	**10.4**	6.7	15.7	**11.6**	7.4	17.4	**27.7**	18.6	39.4	**1.8**	0	26.2	26.2
Romania	**4.9**	2.6	9	**5.4**	2.9	10	**13.9**	7.6	24.4	**60.7**	5.7	96.5	90.8
Slovakia	**22.8**	7.8	50.7	**25.3**	8.7	56.3	**52.5**	21.4	82.5	**0**	0	13.7	13.7
Slovenia	**2**	0.9	4.5	**2.2**	1	5	**5.9**	2.7	12.8	**99**	68.9	100	31.1
Spain	**14.4**	10.1	20.2	**16**	11.2	22.4	**36.7**	27	48	**0**	0	2.2	2.2
Sweden	**0.3**	0.1	1.3	**0.3**	0.1	1.4	**0.9**	0.3	3.8	**100**	99.9	100	0.1
United Kingdom	**3.6**	1.7	7.2	**4**	1.9	8	**10.4**	5	19.9	**86.4**	19.8	99.6	79.8
EU (26 MSs)	**15.6**	**13.6**	**17.9**	**17.3**	**15.1**	**19.9**	**39.2**	**34.9**	**43.7**	**0**	**0**	**0**	**0**
Norway	**0**	0	0.9	**0**	0	1	**0**	0	2.7	**100**	100	100	0
Switzerland	**2.3**	2.3	2.4	**2.6**	2.6	2.7	**6.7**	6.7	7	**98.2**	97.7	98.3	0.6

(a): Baseline survey prevalence of *Salmonella*-contaminated carcasses, called in EFSA's Assessment Methodology Unit's scientific report (EFSA, 2011) observed prevalence and corresponding to the prevalence estimate that accounts for the aspects of clustering but not for imperfect test sensitivity or specificity. The EU prevalence estimate also accounts for weighting.
(b): Lower limit of the 95% CI estimated for (a).
(c): Upper limit of the 95% CI estimated for (a).
(d): True prevalence of *Salmonella*-contaminated carcasses (TCP), estimated using (a).
(e): Lower limit of the 95% CI estimated for TCP, using (b).
(f): Upper limit of the 95% CI estimated for TCP, using (c).
(g): Apparent pooled prevalence (APP), estimated using the TCP from (d) and the characteristics of the test from (d), and taking into account the pooling of samples according to the process hygiene criteria in poultry meat as laid down by Regulation (EC) No 2073/2005.
(h): Lower limit of the 95% CI estimated for APP, using (e).
(i): Upper limit of the 95% CI estimated for APP, using (f).
(j): Probability of meeting the microbiological process hygiene criteria (Prob.) estimated from (g).
(k): Lower limit of the 95% CI estimated for Prob., using (h).
(l): Upper limit of the 95% CI estimated for Prob., using (i).

6. Discussion

Salmonellosis has been the second most frequently reported human zoonotic disease for many years in the EU (EFSA, 2010c). However, among the reported food-borne outbreaks, *Salmonella* has been the most common causative agent, accounting for 1,888 outbreaks in the EU in 2008. Broiler meat and products thereof were reported to be the fifth most frequent cause of these outbreaks, following eggs and egg products, bakery products, pig meat and products thereof and mixed or buffet meals (EFSA, 2010c). The data provided by the Part A report indicated that in many MSs contaminated broiler meat may be an important food-borne source of human *Salmonella* infections, notably of *S.* Enteritidis that is the most commonly reported serovar in human salmonellosis cases but also of *S.* Typhimurium and *S.* Infantis that are also commonly reported in human *Salmonella* infections in the EU (EFSA, 2010c). In addition, the relatively frequent findings of other serovars of public health importance, such as *S.* Hadar, *S.* Virchow, and *S.* Kentucky on broiler meat indicated that broilers are a relevant reservoir for these serovars as well and constitute a potential food-borne source for human infections.

The presence of *Salmonella* on broiler carcasses reflects both surface contamination from faeces and cross-contamination during slaughter (between infected and non-infected slaughter batches) from the processing equipment and the processing environment at the slaughterhouse (Corry et al., 2002; Rasschaert et al., 2008). Following slaughter of a *Salmonella*-positive broiler batch, unless effective cleaning is undertaken, *Salmonella* can persist in the slaughterhouse environment and contaminate subsequent slaughter batches and result in *Salmonella* contaminated broiler meat being placed on the market.

6.1. Context of *Salmonella* baseline survey

This EU-wide BS estimated the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses within 26 MSs and two non-MSs and these estimates were published in the Part A report. Two Part B reports were prepared regarding the survey. A first report Part B, which was published on 5 August 2010, provided the additional EU level *Campylobacter* analyses results. The second, present, Part B report presents the additional analyses with regard to *Salmonella*-contaminated broiler carcasses.

During the conduct of the survey, some compulsory complementary data were recorded pertaining to the broilers sampled, the slaughterhouse involved and the subsequent handling of samples. The pragmatic choice as to which potential risk factors for which data should be collected was made by the MSs, partly based upon EFSA's proposal for the survey design (EFSA, 2007a). This Part B report considers whether any of these factors can be associated with the presence of *Salmonella* on broiler carcasses. It should be noted that many potential factors of relevance to the *Salmonella* contamination of carcasses such as – among others – the presence of other farm animals in the holding, hygiene during slaughter and subsequent processing, slaughter techniques, the speed of the slaughter line and the cleaning and disinfection procedures used, were not a part of the present survey. Also, while the *Campylobacter*-subsurvey involved sampling at two points along the slaughterline: at the slaughterhouse in-point where batches were sampled, and at the out-point, just after chilling, where carcasses were sampled; the *Salmonella*-subsurvey only analysed the carcass samples, and therefore no data for *Salmonella* were collected on the prevalence of *Salmonella*-infected batches, as was the case for *Campylobacter*. Furthermore, sampling was performed to broadly represent the production methods present in participating countries, so the number of broilers sampled from minority production systems was low. For example, 90% of sampled batches originated from conventionally-reared (i.e. housed) birds and about 70% of the chilling methods used for the batches were 'air'. Thus, the BS dataset was unbalanced with respect to certain categories of risk factors. Consequently, the power of subsequent analyses was hampered by these small quantity of data in these sub-populations, resulting in an inability to eliminate chance as the cause of findings, which is important for the interpretation of the results of the associated factor analyses. As such, the present report does not aspire towards a comprehensive analysis of all risk factors believed to impact on *Salmonella* risk, but an assessment of, with reasonable confidence, those factors for which information was captured in this survey by a questionnaire indicated an association with *Salmonella* positivity.

In some instances the information analysed pertained to production methods e.g. outdoor access production systems or previous thinning in the flock. Association of such management practices might reasonably be regarded as a 'risk factor' indicating the potential to produce broilers in an alternate way to manage risk. In other instances the information pertains to the sensitivity of the sampling protocols, e.g. the time (hours) between sampling and analysis, and while not strictly risk factors, these analytical outcomes might be useful information when designing and interpreting national monitoring programmes.

MSs could also report other optional information on a voluntary basis, but these data were too scarce to enable an epidemiological analysis within the scope of this Part B report.

MSs should consider the information available in this report as an adjunct to the understanding of the nature of the *Salmonella* problem in broilers. These outcomes may inform national control programmes or subsequent in-depth research.

6.2. Analysis of factors associated with *Salmonella*-contaminated broiler carcasses

In the EU level multivariable regression analyses (including the two non-MSs), the structure of the statistical models took into account the fact that broilers originating from the same country have a higher probability of sharing similar domestic conditions, and that broiler carcasses from the same slaughterhouse were submitted to similar processes. Furthermore, possible country-confounding effects were also accounted for in the analyses.

Additional analyses performed at the level of the two country groups in this survey (i.e. MSs having a low or high *Salmonella* prevalence) allowed the assessment of the variability of effects of reported factors between these country groups. However, these analyses should still be regarded as indicative and need to be complemented by specific studies carried out at national level taking into account domestic conditions.

It is also worth addressing the interpretation of findings where an association was not found in the analyses. The statistical methods used are able to provide a robust answer to the question of whether the studied variable is associated with prevalence in the dataset. A statistically significant conclusion indicates that the outcome of association would have been extremely unlikely to have arisen by chance. However, in some instances where no statistical significance was observed, trends of association would appear to be present, but chance occurrence of random events could not be discounted from the trends in the dataset. This absence of a statistically significant result should not be understood to disprove association, merely the inability to rule out chance and therefore the absence of mention in the final model as an associated factor should be interpreted in this context.

6.2.1. Effect of factors on the risk of *Salmonella* contamination of broiler carcasses

The present BS studied eight potentially associated factors: flock production type, previous thinning in the flock, age (days) of broilers at slaughter, quarter (of the year 2008) of sampling, time (hour) of sampling during the day, time (hours) between sampling and testing, capacity of the slaughterhouse, type of chilling method, and the result of *Campylobacter* based on broiler carcass samples.

Besides significant differences between the countries, the analyses of the survey results showed that two factors were significantly associated at EU level with *Salmonella* contamination of broiler carcasses; the risk of contamination with *Salmonella* increased with the capacity of the slaughterhouse and with processing the carcass later during the day.

Salmonella is a known common inhabitant of the intestinal tract of broilers. Thus, the organism can be expected to contaminate broiler carcasses during the slaughtering process as a result of faecal contamination. Since broiler carcasses are a foodstuff on which the skin of the animal remains, there also exists the potential for carry-over of pre-existing faecal contamination of the skin (e.g. from

contaminated faeces on crates during transportation to the slaughterhouse) through the slaughter process.

The prevalence of *Salmonella*-contaminated broiler carcasses increased when the carcasses were processed later during the day. It could be that carcasses sampled later during the day were contaminated from *Salmonella*-infected batches slaughtered earlier during the same day with environmental accumulation of contamination in the slaughterhouse environment (cross-contamination). This indicates the need to investigate the mechanism and relevance, in terms of the level, of any accumulation of contamination as well as the potential efficacy of slaughterhouse-specific hygienic procedures and or other interventions keeping in mind that *Salmonella* can grow in the slaughterhouse environment. However, another explanation for the higher prevalence later during the day could be that known infected batches were slaughtered later during the day (logistic slaughtering).

The observed association between the *Salmonella*-contamination result on the broiler carcass and the capacity of the slaughterhouses indicates that a higher throughput of broiler carcasses seemed to put carcasses at risk of being contaminated. This observation is supported by a previous study based on results from the Dutch *Salmonella* monitoring programme (Fels-Klerx et al., 2008). This may be due to the decreased opportunity for cross-contamination at the smaller plants, as they work at a slower pace and with fewer birds and/or flocks. Another reason could be that in larger slaughterhouses more flocks are combined into one batch, which may increase the cross-contamination.

However, as mentioned above, many potential slaughterhouse-level factors of relevance to the *Salmonella* contamination of carcasses were not part of the present survey. The presence of such confounding factors warrants caution while interpreting the associations found.

The EU level multivariable analyses did not show an association between the *Salmonella*-contamination result on the broiler carcass and flock production type, quarter of sampling, hours between sampling and testing, type of chilling, *Campylobacter* result on broiler carcasses, age of broilers, and previous thinning in the flock. As explained above, the absence of a statistically significant result, at EU level, should not be understood to totally disprove association; merely the inability to rule out chance and therefore the results should be interpreted with caution.

At flock level, the age of broilers and the month of sampling were earlier found to be associated with *Salmonella* prevalence in the survey of broiler flocks (EFSA, 2007e). Flocks with younger birds were associated with a higher risk of being *S.* Enteritidis-positive but for the other serovars (*S.* Infantis, serovars other than *S.* Enteritidis and *S.* Infantis) there was no association with the age of the broilers. The prevalence of intestinal contamination by *Salmonella* in broiler flocks has been reported to decrease with the birds' age in other studies (Bailey and Cox, 1991); and resistance of older birds to *Salmonella* spp. infection might be explained by a natural antagonist digestive flora in caecum and colon (Nurmi and Rantala, 1973). The analysis of the 2005-2006 broiler flock prevalence also suggested an effect of the month of sampling on the flock prevalence for all studied *Salmonella* serovars or serovar-groups and broiler flocks sampled during late autumn and winter of 2005-2006 were generally characterised by a higher risk of *Salmonella* infection at Community level. In the present carcass survey, no seasonal effect on the risk of *Salmonella* contamination of broiler carcasses samples was observed, but the presence of uninvestigated slaughterhouse management procedures could have masked such existing association at batch level.

Delaying the time between sampling and testing did not increase the risk of detecting *Salmonella* from the broiler carcasses and this observation might indicate that testing procedures were robust. Also, at EU level, no significant difference was disclosed between conventional broiler flock production types and the other production types. This is consistent with the findings of the 2005-2006 broiler survey were the flock prevalence for *S.* Enteritidis and for serovars other than *S.* Enteritidis and *S.* Infantis was not different between flock production types (conventional and non- conventional ones).

The EU level multivariable analyses did not show an association between the *Salmonella*-contamination result on the broiler carcass and the type of chilling. However, there were indications that the risk of *Salmonella* contamination of carcasses was lower in the case of broiler carcasses chilled by a mixed-chilling method in the subset of countries with a carcass prevalence below 6.4%. Mixed chilling methods included processes with more than one chilling method and such combined chilling methods, on average, might result in less cross-contamination/redistribution of *Salmonella* between carcasses or tended to lower cross-contamination. This likely diverging outcome in factors identified in datasets at EU level and country-group level indicated that risk factors for *Salmonella* contamination of carcasses are likely to change according to prevalence situations, whether low or high. In general, broiler carcasses should be chilled as quickly as possible to limit the growth of microorganisms on the carcasses.

There was no evidence of association between the results of *Campylobacter* and *Salmonella* testing based on broiler carcass samples, despite the fact that both represent faecal contamination of carcasses. This might reflect that the prevalence of *Salmonella*-contaminated carcasses was not importantly influenced by factors favouring cross-contamination or spread of both bacteria. This result probably reflects the different epidemiology and ecology of these organisms, for example differences in transmission routes to the broiler flocks on farm. Therefore, it is important to realise that meaningful and effective *Salmonella* control programmes may not necessarily imply that these programmes are effective in controlling *Campylobacter*.

6.2.2. Effect of the slaughterhouse on the risk of *Salmonella* contamination of broiler carcasses

The effect of the slaughterhouse on carcass contamination was also considered in the analyses. The results showed that the baseline risk of *Salmonella* carcass contamination varied significantly between countries and between slaughterhouses within a country, even when other factors, such as time (hour) of sampling and capacity of the slaughterhouse, were taken into account in the statistical model. Thus, there were slaughterhouses (within a country) with a higher prevalence and slaughterhouses with a lower prevalence of *Salmonella*-contaminated carcasses.

As mentioned above, many potential factors of relevance to the *Salmonella* contamination of broiler carcasses were not a part of the present survey. Slaughterhouse-specific factors with a potential effect on the risk of *Salmonella* contamination of carcasses, that might explain the observed heterogeneity between slaughterhouses, could relate to the within-batch *Salmonella* prevalence in the (incoming) slaughter batches or to the bacterial load of the broiler intestinal and faecal contents. Moreover, other slaughterhouse effects might relate to slaughter hygiene practices impacting on the extent to which intestinal and faecal contents contaminate carcasses. In this context the analyses showed that 46% of the unexplained variance in *Salmonella* prevalence might have been attributable to slaughterhouse-specific factors for which no data were gathered during the survey.

For countries with prevalence below the EU median the baseline risk of *Salmonella* carcass contamination did not vary between the slaughterhouses. This could indicate that when prevalence is generally low the slaughterhouse-specific effects, such as impact of process methods, are weaker. For countries with prevalence above the EU median the baseline risk of *Salmonella* carcass contamination varied between the slaughterhouses. In this latter subgroup, 49% of the unexplained variance might have been attributable to slaughterhouse-specific factors for which no data were gathered during the survey. It may be in the interest of MSs to study further these uninvestigated slaughterhouse-specific factors in their country in order to improve the control of *Salmonella* and the protection of public health.

As mentioned above, while the *Campylobacter*-subsurvey involved sampling at two points along the slaughterline – at the slaughterhouse in-point where batches were sampled and at the out-point, just after chilling, where carcasses were sampled – the *Salmonella*-subsurvey only analysed the carcass samples, and therefore no data were collected on the prevalence of *Salmonella*-infected incoming batches. However, the Part A report reported a significant correlation between the 2005 to 2006 BS

prevalence results of *Salmonella* in broiler flocks (EFSA, 2007c) with the 2008 prevalence results of *Salmonella*-contaminated broiler carcasses indicating that lower broiler flock *Salmonella* prevalence translate into lower prevalence of *Salmonella*-contaminated carcasses.

6.2.3. Analysis of the *Salmonella* serovars distribution

The most commonly reported *Salmonella* serovars in the survey were *S.* Infantis, *S.* Enteritidis and *S.* Typhimurium. The distribution of the serovars varied among MSs. Although there was a concentration of most *S.* Infantis isolates in Hungary, and a high serovar-specific prevalence observed in this country, *S.* Infantis was still the most widely-distributed serovar being reported in 15 countries. Thus its presence is not a local phenomenon. *S.* Enteritidis was present in 14 countries, and its prevalence distribution shows a predominance of higher values than other serovars (being the dominant serovar in five MSs), confirming its role as the most important serovar found in broilers in Europe. *S.* Typhimurium was less frequently reported compared to *S.* Kentucky but was more spread across Europe, because it was isolated in 10 countries while *S.* Kentucky was found only in six. *S.* Agona and *S.* Mbandaka were also widely distributed (10 countries), although at a lower prevalence.

At EU level the majority of the *Salmonella* serovars isolated from broiler carcasses have also been isolated from flocks with broilers and laying hens, suggesting the existence of common sources of *Salmonella* infection in poultry production and/or that transmission between the two production types may occur. For many MSs the current serovars appear to have been established in the broiler production for some time. Feed may have played a role in introducing a part of these infections, as nearly all serovars in broilers have also been detected in poultry feed, feed mills or feed raw materials. Feed should therefore be regarded as an important source for the introduction of new *Salmonella* serovars that may be able to establish themselves in poultry holdings for a shorter or longer period and constitute a risk for human health. Further studies are necessary to elucidate the importance of feed in the *Salmonella* transmission.

Despite the fact that the BSs in broiler flocks and on carcasses were conducted two years apart, there seemed to be an overall agreement on the presence and distribution of serovars when comparing flocks and carcasses at MS level suggesting that many serovars have become well-established in national broiler productions. A few exceptions include Ireland and Cyprus, which had a complete change of profile from one survey to another. In Cyprus, these changes are observed even when considering a wider range of serovars than the ones chosen as the focus of this report. This change could be due to surveillance and control activities, or the importing of broilers from countries with a predominance of different serovars. The change in Ireland is more visual but appears more severe than is actually the case.

The *Salmonella* serovars present in broiler carcasses show a relatively fair correlation with the serovars found in humans. Some prevalent serovars in broiler carcasses, such as *S.* Enteritidis, *S.* Typhimurium and *S.* Infantis have been and continue to be implicated in human diseases. Studies on source attribution suggest that broiler meat is one of the most important sources of human salmonellosis in Europe, although the relative importance when compared to other food sources depends on serovar epidemiology and consumption habits in each region or country (Pires et al., 2010). The survey results seem to support these observations, given the overall predominance of the same serovars in broiler carcasses, broiler flocks and humans. However, *S.* Mbandaka, which appears as one of the most frequently observed serovars among broiler flocks and carcasses, does not seem to have any major impact on human health.

6.3. Simulation-based assessment of *Salmonella* process hygiene criteria in poultry meat

Using different scenarios in a simulation-based assessment it was possible to relate prevalence of *Salmonella*-contaminated carcasses to the probability of meeting the *Salmonella* process hygiene criteria set by EU legislation and *vice versa*. Given the simulation model used and the assumptions made it appeared that only countries with low to very low prevalence of *Salmonella*-contaminated

broiler carcasses would meet the *Salmonella* microbiological process hygiene criteria in poultry meat with reasonable certainty. However, important differences were observed in the certainty of the MS-specific estimations of the probability of meeting those criteria. Given the basic simulation approach, which was the same for all MSs, these differences related to the width of the MS-specific confidence intervals around the baseline survey prevalence estimates.

CONCLUSIONS

This second Part B survey report provides results from further analyses of the BS on *Salmonella* on broiler carcasses at slaughterhouse level. These are results regarding the association of eight batch and/or slaughterhouse level factors, on which data were collected, and *Salmonella* contamination of carcasses. In addition, further analyses of the distribution of the isolated serovars of *Salmonella* across the EU are included in the current report. Lastly, results are reported from a simulation-based assessment of the probability of meeting the *Salmonella* process hygiene criterion in poultry meat.

- The risks for contamination of broiler carcasses with *Salmonella* varied significantly between countries and between slaughterhouses within a country even when other associated factors, such as time (hour) of sampling and slaughter capacity of the slaughterhouse, were accounted for. Thus, there were slaughterhouses with a higher prevalence and slaughterhouses with a lower prevalence of *Salmonella*-contaminated carcasses within a country.
- At EU level, the risk of *Salmonella* contamination of carcasses increased with the slaughter capacity of the slaughterhouse and with processing of the carcass later during the day.
- Additional analyses performed for countries with low or high prevalence of *Salmonella*-contaminated carcasses, respectively, using the median of prevalence (6.4%) as the cut-off point, showed that there was a variation between the low and high prevalence countries in factors indicated to be associated. For the low prevalence group the chilling method of the carcasses was indicated to be associated with the prevalence of *Salmonella*-contaminated carcasses and carcasses chilled by mixed methods appeared to be at lower risk. For the higher prevalence country group, there were indications that the risk of *Salmonella* contamination of carcasses increased with the slaughter capacity of the slaughterhouse and with the processing of the carcass later during the day.
- The analyses showed that 46% of the unexplained variance in *Salmonella* contamination of carcasses might have been attributable to slaughterhouse-specific factors influencing the prevalence of *Salmonella* contamination of carcasses but for which no data were gathered during the survey. It was not possible to estimate the association of these factors with *Salmonella* contamination of broiler carcasses and their potential confounding role on the effect of factors on which data were available.
- The magnitude of the unexplained variance in *Salmonella* contamination of carcasses was bigger for the group of countries with *Salmonella* prevalence above the EU median compared to the group of countries with prevalence below the EU median. For countries with prevalence below the EU median the baseline risk of *Salmonella* carcass contamination did not vary importantly between the slaughterhouses.
- Even though the BS on broiler flocks and carcasses were conducted two years apart, there seemed to be an overall agreement on the presence and distribution of different *Salmonella* serovars when comparing flocks and carcasses at MS level, which suggests that many of the serovars have become well-established in the broiler production in the countries.
- The *Salmonella* serovars present on broiler carcasses show a relatively fair correlation with the serovars found in human salmonellosis cases supporting the results of recent source attribution studies and the general belief that broiler meat is one of the most important sources of human salmonellosis in Europe, although the relative importance when compared to other food sources depends on serovar epidemiology and consumption habits in each region or country.

RECOMMENDATIONS

- MSs are invited to consider the factors found to be associated, at EU level, with *Salmonella*-contaminated broiler carcasses when they are designing and implementing national *Salmonella* control programmes for broiler meat.
- MSs are specifically encouraged to verify the food business operators' own controls for *Salmonella* in their slaughterhouses in order to prevent subsequent contamination of broiler carcass and to improve protection of public health, since the slaughter process was shown to have an impact on the risk of carcass contamination.
- Further national studies are recommended to identify more closely the factors that put broiler carcasses at risk of becoming contaminated with *Salmonella* in a country, taking into account the national *Salmonella* prevalence and the characteristics of the national broiler production, including slaughter procedures.

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LIST OF APPENDICES

A. STATISTICAL METHODOLOGY USED IN THE ANALYSIS OF THE REPORT PART B PRODUCED FOR THE EU-WIDE BASELINE STUDY ON THE PREVALENCE OF *SALMONELLA* ON BROILER CARCASSES

1. Data import and management

All data management and statistical analyses in this report were performed using SAS, whereas figures were constructed using R.

The data provided by EFSA contain information at slaughterhouse sample level.

2. Methodology and tools for descriptive analysis

The descriptive section presents a thorough description of the samples by all independent variables. This descriptive analysis is based on boxplots, barplots, scatter plot frequency tables, as well as some tests to establish the association between the risk factor and the result of *Salmonella*. Note that these results should be interpreted only within the context of an exploratory analysis. Further analyses using appropriate modelling techniques should be used to validate these results in their proper context.

Hereafter, some detailed discussion is provided on the tests used to study the association between *Salmonella* prevalence and the independent variables. Note that this association is studied using data at EU level, that is including MSs and two non-MSs.

Chi-square tests

Consider two categorical variables *X* and *Y*, *X* having *I* levels and *Y* having *J* levels. The *IJ* possible combinations of outcomes can be displayed in a contingency table having *I* rows for the categories of *X*, and *J* columns for the categories of *Y*. The null hypothesis H_0 of independence is equivalent to cell probabilities satisfying $\{\pi_{ij} = \pi_{i+}\pi_{+j}\}$. For a sample of size *n* with cell counts $\{n_{ij}\}$, the values $\{\mu_{ij} = n\pi_{ij}\}$ represent the expected frequencies, i.e. the values of the expectations $\{E(n_{ij})\}$ when H_0 is true. The sample cell counts can then be compared to the expected frequencies to judge whether the data contradict H_0. If the null hypothesis is true, then n_{ij} should be close to μ_{ij} in each cell. The larger the differences $\{n_{ij} - \mu_{ij}\}$, the stronger the evidence against H_0. In practice, μ_{ij} can be estimated by $\hat{\mu}_{ij} = n_{i+}n_{+j}/n$.

A test statistic which uses this property is the Pearson Chi-squared statistic, given by:

$$X^2 = \sum \frac{(n_{ij} - \hat{\mu}_{ij})^2}{\hat{\mu}_{ij}},$$

which is asymptotically distributed according to a Chi-square with $(I-1)(J-1)$ degrees of freedom.

It is difficult to evaluate whether the available sample size is large enough for these asymptotic results to be valid. In general this is given by $\{\mu_{ij} \geq 5\}$. When the sample size is small, one can resort to inferences using *exact* distributions rather than large sample approximations (Agresti, 2002).

Spearman correlation

Also called Spearman Rank Order correlation (Spearman, 1904), this is a nonparametric measure of association based on ranks of data values. It is noted by:

$$\theta = \frac{\sum_i \left((R_i - \bar{R})(S_i - \bar{S})\right)}{\sqrt{\sum_i (R_i - \bar{R})^2 (S_i - \bar{S})^2}}$$

R_i denotes the rank of the *i*th observation of the first variable, S_i denotes the rank of the *i*th observation of the second variable, and $\overline{R}$ and $\overline{S}$ are the means of the ranks for each of the variables. This statistic can be used when the variables are ordinal.

Cochran-Mantel-Haenszel Chi-square test for linear trend

The Cochran-Mantel-Haenszel Chi-square statistic (Mantel-Haenszel, 1959) tests the alternative hypothesis of there being a linear association between the row variable (the response) and the column variable (the risk factor). Both variables must lie on an ordinal scale. The Mantel-Haenszel Chi-square statistic is computed as:

$$Q_{CMH} = (n-1)\rho^2$$

where ρ^2 is the Pearson correlation between the row variable and the column variable. The Pearson correlation and thus the Mantel-Haenszel Chi-square statistic use the Ridit scores, which are defined as rank scores standardised by the sample size (Bross, 1958). Under the null hypothesis of no association, Q_{CMH} has an asymptotic Chi-square distribution with one degree of freedom. This test is more powerful than the Chi-square test, because it takes into account the ordinal nature of the variables.

3. Methodology and tools for the regression analysis

The hierarchical structure in the data can essentially be expressed as follows: broiler carcasses within a slaughterhouse, and slaughterhouses within a country. Interest goes to prevalence in broiler carcasses. Therefore, let π_i be the probability for a sample to be positive, let n_{ij} be the number of samples in slaughterhouse *j* from country *i*. The starting point for inference on the 'sample level prevalence' of the different outcome variables is the binomial distribution for the number of positive broilers y_{ij} in slaughterhouse *j* from country *i*:

$$y_{ij} \sim Bin(n_{ij}, \pi_i). \quad (1)$$

In a fully random sample these numbers y_{ij} could be combined in a straightforward way to estimate the prevalence for country *i*. The main complications here are:

1) the assumptions on the binomial distribution are violated; and
2) the sample is not drawn at random (but essentially stratified).

Indeed,

- *violation of independence*: outcomes from the same slaughterhouse are expected to be more alike (correlated) as compared to outcomes from a different slaughterhouse (hierarchical correlation structure);
- *violation of constant probability*: samples, even from the same slaughterhouse, might have different probabilities of being infected (heterogeneity of probability).

Clustering

To account for the possibility of samples from the same slaughterhouse being more alike than from different slaughterhouses, there exist, broadly, three approaches:

- *ignore the correlation:* while this typically leaves the consistency of point estimation intact, the same is not true for measures of precision. In case of a "positive" correlation (i.e. samples within a slaughterhouse are more alike than between slaughterhouses), then ignoring this aspect of the data, just as ignoring overdispersion, overestimates precision and hence underestimates standard errors and widths of CIs;

- *account for correlation*: the existence of correlation is recognised but considered as a nuisance characteristic. A crude way of correcting for clustering is done by computing a so-called *design effect*. Roughly, the design effect is a factor comparing the precision under simple random sampling with the precision of the actual design. Standard errors, computed as if the design had been simple random sampling, can then be artificially inflated using the design effect; and
- *model correlation:* in contrast to the previous view-point, one can have a genuine scientific interest in the correlation itself. The intra-class correlation should be addressed in order to obtain valid statistical inferences, and specialised methods which model the correlation, should be used.

Obviously the third method is much broader. Hence, analysis strategies consistent with an interest in the intra-cluster dependence can be applied. There exist two important families of models which can be used for this purpose: random effects models and marginal models.

In a *marginal* or *population-averaged model*, marginal distributions are used to describe the outcome vector $\boldsymbol{Y}$, given a set X of predictor variables. A marginal model can be used to evaluate the overall (or population-averaged) trend as a function of covariates. Alternatively, in a random effects model, also called cluster-specific models or multilevel models, the predictor variables X are supplemented with a vector $\boldsymbol{u}$ of random effects (specific to the cluster/slaughterhouse), conditional upon which the components of $\boldsymbol{Y}$ are usually assumed to be independent. Thus, cluster-specific models are differentiated from population-averaged models by the inclusion of parameters which are specific to the cluster/slaughterhouse. In random effects models, the intra-cluster correlation is assumed to arise from natural heterogeneity in the parameters across clusters (slaughterhouse).

There are two routes to introduce randomness into the model parameters. The first approach introduces the random effects on the probability scale, such as the beta-binomial model (Skellam, 1948). The second approach introduces random effects in the linear predictor, yielding the classical mixed-effects models (Stiratelli, Laird and Ware, 1984). A random effects logistic regression model is an example of the second approach, where it is assumed that the number of positive samples y_{ij} in slaughterhouse *j* in country *i* follow a binomial distribution:

$$y_{ij} \sim Bin(n_{ij}, \pi_{ij}), \quad (1)$$

with a mean modelled through a linear predictor containing fixed regression parameters β_i and slaughterhouse-specific parameters u_{ij}:

$$\text{Logit}(p_{ij}) = \beta_i + u_{ij}.$$

It is assumed that the slaughterhouse-specific effects are normally distributed with a mean of zero and some variance σ_i^2, i.e. $u_{ij} \sim N(0, \sigma_i^2)$. The above model can be interpreted as a logistic mixed effects model for each slaughterhouse, where some of the regression parameters are specific (random effects), while others are not (fixed effects). The random effects u_{ij} express how unit-specific trends deviate from the population-averaged trends.

In addition to the slaughterhouse-specific random intercepts, possible risk factors are taken into account, which are given by categorical, ordinal and continuous variables. It was decided not to use a global intercept, but rather allow the random intercept explain the slaughterhouse effect and the covariates show the possible influences on the *Salmonella* result.

In order to select the best subset of risk factors, both the forward and the backward selection were used. Forward selection starts with no predictors at all and then sequentially adds into the model the predictor that most improves the fit. Supposing the current model contains the predictors represented by parameter estimates $\hat{\beta}$, and a predictor is added, this would result in a matrix of estimable functions *L*; it is assumed that the matrix *Q* depends on the estimation method. Thus, the improvement in the fit is based on the type III test of fixed effects, based on the following *F* statistic:

$$F = \frac{\hat{\beta}'L'(LQL')^{-1}L\hat{\beta}}{\text{rank}(LQL')}.$$

A typical strategy adds in sequentially the predictor producing the largest value of *F*, stopping when no predictor produces an *F*-ratio greater than the 95th percentile of the $F_{1,N-k-2}$ distribution. Instead, backward selection starts with the full model and sequentially deletes predictors. Like forward selection, it used the *F*-ratio to choose the predictor for deletion: in this case, the predictor producing the smallest *F*-ratio at each stage was dropped, stopping when each predictor in the model produces an *F* greater than the 95th percentile of the $F_{1,N-k-2}$ distribution when dropped.

Unlike for correlated Gaussian outcomes, the parameters of the cluster-specific and population-averaged models for correlated binary data describe different types of effects of the covariates on the response probabilities (Neuhaus, 1992). The choice between population-averaged (i.e. marginal models) and cluster-specific (i.e. mixed effects models) strategies may heavily depend on the scientific goals. Population-averaged models evaluate the overall risk as a function of covariates. With the cluster-specific approach, the response rates are modelled as a function of covariates and parameters, specific to a slaughterhouse. In such models, the interpretation of fixed-effect parameters is conditional on a constant level of the slaughterhouse-specific parameter (e.g. random effect). Diggle et al. (1994; 2002) recommended the random effects model for inferences about individual responses and the marginal model for inferences about margins, that is, the objectives (or the types of inferences) in a study should determine which suitable statistical model to use. For more details, see e.g., Aerts et al. (2002) and Molenberghs and Verbeke (2005).

Weighting

Most statistical procedures analyse the data as if they were collected as a simple random sample. As a result, these procedures may lead to biased estimates and may underestimate the variability present in the data, when the data actually arise from complex surveys. Assigning weights to the observations is one possible approach to correct for the differences between the complex survey design and simple random sampling. In general, weights are used to try to 'reconstruct the total population', in order to avoid that certain strata or subpopulations are over- or under-represented. The weighting scheme for broiler carcasses is set out below.

Ideally, in order to calculate the weights, two pieces of information should be taken into account, first the probability of selection of a slaughterhouse within a country, and second, given that a slaughterhouse is selected, the probability of selecting a specific sample within a slaughterhouse.

For the first probability the total number of slaughtered broiler carcasses within the country and the number of slaughtered broilers in the slaughterhouses included in the survey should be considered. To calculate the second probability, the number of slaughtered broilers per year in each slaughterhouse could be used. However, the capacity of the selected slaughterhouses is given in the survey as an ordinal variable categorised in big ranges (for instance, 1,000,000-4,999,999, 5,000,0000-9,999,999 or ≥10,000,000). Thus, using this to calculate the second probability, for the generation of weights, more bias might be introduced, considering the wide ranges of the categories for this variable. Thus, only the first probability was opted for use for, when calculating weights for broiler carcasses.

Note that the total number of slaughtered broilers within a country is provided by the variable "V055_SlaughPop", whereas the number of samples can be calculated from the data.

Finally, it is observed that the sum of these weights gives an indication of the total number of slaughtered broilers *N* in the EU. To avoid overemphasising the importance of the broilers used in the sample, the standardisation of calculated weights is therefore needed so that they sum up to N_s, i.e. the sample size. In general, this implies that, for broiler/batch *k*, in slaughterhouse *j*, in country *i*:

$$\text{if } \sum_{ikc} w_{ijk} = N \text{ then } \sum_{ijk}(N_s/N)w_{ijk} = N_s.$$

Therefore, standardised weights $w^*_{ijk} = (N_s/N)w_{ijk}$ will be used. For this report, the adjustment includes 28 countries, Greece did not participate in the BS and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

Multicollinearity analysis among risk factors

A formal method to detect multicollinearity is given by the VIF. This factor measures how much the variances of the estimated regression coefficients are inflated as compared to when the predictor variables are not linearly related. Essentially, each risk factor X_k is regressed on the other risk factors X in the model. The corresponding coefficient of multiple determination R^2_k is then used to calculate the VIF:

$$VIF_k = (1 - R^2_k)^{-1}$$

Note that the VIF is equal to 1 when $R^2_k = 0$, i.e. when X_k is not linearly related to the other risk factors. When $R^2_k \neq 0$, then the VIF will be larger than 1, indicating an inflated variance for the estimated regression coefficients due to correlations among the risk factors. A maximum VIF exceeding 10 is frequently interpreted as an indication of multicollinearity.

For the categorical covariates, the VIF can be calculated in a similar way using:

$$R^2 = 1 - \exp\{2[\log L(M) - \log L(0)]/n\},$$

with $\log L(M)$ and $\log L(0)$ representing the maximised log-likelihoods for the fitted model and the "null" model, containing only the intercept, and *n* referring to the sample size (Neter et al., 1996; Agresti, 1996).

Intra-cluster Correlation Coefficient

This is a measure to describe the similarity of the responses to the outcome within a slaughterhouse (cluster). For a random intercept model, the ICC (or intra-slaughterhouse correlation) is considering the variance of the random intercepts and the variance of the standard logistic density (Molenberghs and Verbeke, 2005). The ICC was approximated as the ratio of the variance of the random effects and the sum of the variance of the random effects and the variance of the standard logistic density. The ICC ranges between zero and one and corresponded respectively to scenarios of low (closer to zero) or high (closer to one) proportions of unexplained variance that was due to random effects (slaughterhouse-specific effects, between-slaughterhouse variability). Let *z* be a matrix of estimable functions and *D* be the unstructured variance-covariance matrix of the random effects b_i. Thus, the ICC is given by the following formula:

$$ICC = \frac{z'Dz}{z'Dz + \pi^2/3}.$$

B. DESCRIPTIVE ANALYSIS OF POTENTIAL FACTORS ASSOCIATED WITH *SALMONELLA*-CONTAMINATED BROILER CARCASSES

Flock production type

Table 8 reports the numbers and percentages of broiler carcasses sampled in the EU per flock production type. Figure 9 displays the barplot of the prevalence of *Salmonella*-contaminated broiler carcasses by flock production type showing that the prevalence is similar between the known production types, whereas it is significantly higher when the flock production type is unknown. The *P*-value of the Pearson Chi-square statistic (Table 9) is smaller than 0.05, thus there is association between the prevalence of *Salmonella*-contaminated broiler carcasses and the flock production type, even though Figure 9 indicates that this association is totally driven by the "unknown" production type. A graphical display of the distribution of positive and negative broiler carcass samples collected by country and in the EU per flock production type is presented in Figure 10.

Table 8: Number and percentage of sampled broiler carcasses by flock production type in the EU [(a)] (based on 28 countries), 2008

Flock production type	EU	
	N	%
Conventional	9,152	*91.2*
Free-range organic	112	*1.1*
Free-range standard	641	*6.4*
Unknown	130	*1.3*
Total	10,035	*100.0*

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis. Estonia reported all flocks as free-range standard, but broilers were kept inside with no outdoor access and these data are included in the analysis.

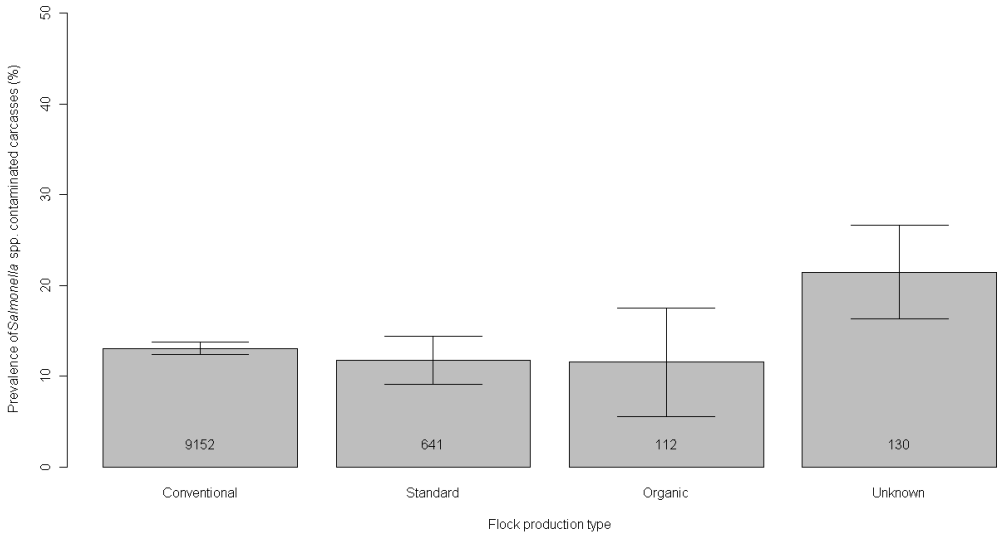

Figure 9: Prevalence of *Salmonella*-contaminated broiler carcasses by flock production type in the EU [(a)] (based on 28 countries), 2008

Note: The numbers appearing within the shaded areas of the bars indicate the total number of sampled broiler carcasses for each category.

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis. Estonia reported all flocks as free-range standard, but broilers were kept inside with no outdoor access and these data are included in the analysis.

Table 9: Pearson's Chi-square to test for the independence between flock production type and *Salmonella* contamination result on the broiler carcass

	Chi-square statistic (*P*-value)
Salmonella spp.	16.09 (0.0011)

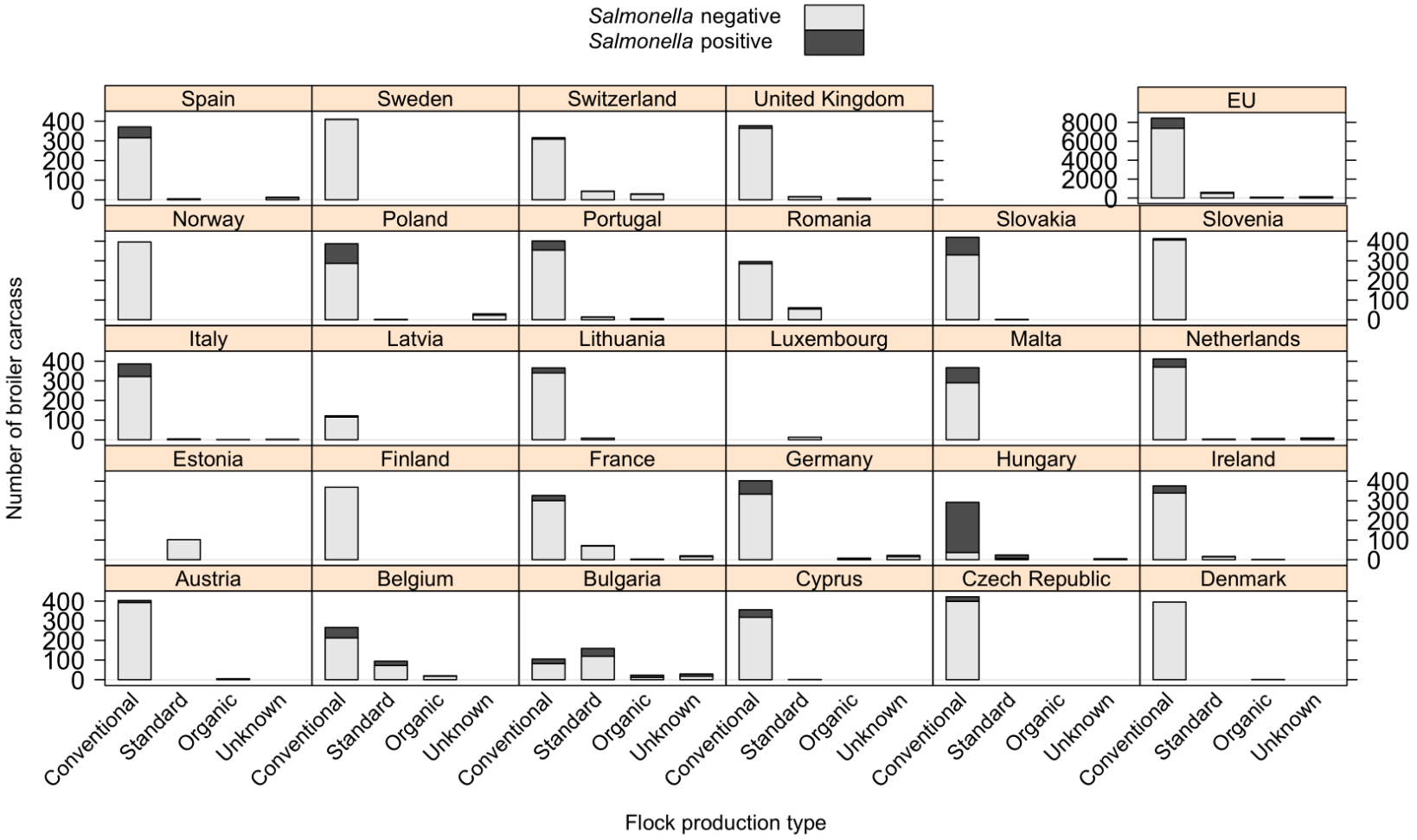

Figure 10: Distribution of *Salmonella*-contaminated broiler carcasses by flock production type, by country and in the EU [(a)], 2008

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis. Estonia reported all flocks as free-range standard, but broilers were kept inside with no outdoor access and these data are included in the analysis.

Previous thinning in the flock

Table 10 reports the numbers and percentages of broiler carcasses contaminated with *Salmonella* in the EU per previous thinning in the flock. Figure 11 displays the barplot of the prevalence of *Salmonella*-contaminated broiler carcasses by previous thinning in the flock showing that the prevalence is independent from previous thinning, even though the samples for which the thinning status is unknown present a higher prevalence. The *P*-value of the Pearson's Chi-square statistic (Table 11) is smaller than 0.05, thus there is association between the prevalence of *Salmonella*-contaminated broiler carcasses and previous thinning in the flock, even though Figure 11 indicates that this association is totally driven by the"unknown" status of the previous thinning. A graphical display of the distribution of positive and negative broiler carcass samples collected by country and in the EU per previous thinning status is presented in Figure 12.

Table 10: Number and percentage of sampled broiler carcasses by previous thinning in the EU [(a)] (based on 28 countries), 2008

Previous thinning in the flock	EU	
	N	%
No	5,712	*56.9*
Unknown	1,564	*15.6*
Yes	2,759	*27.5*
Total	10,035	*100.0*

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

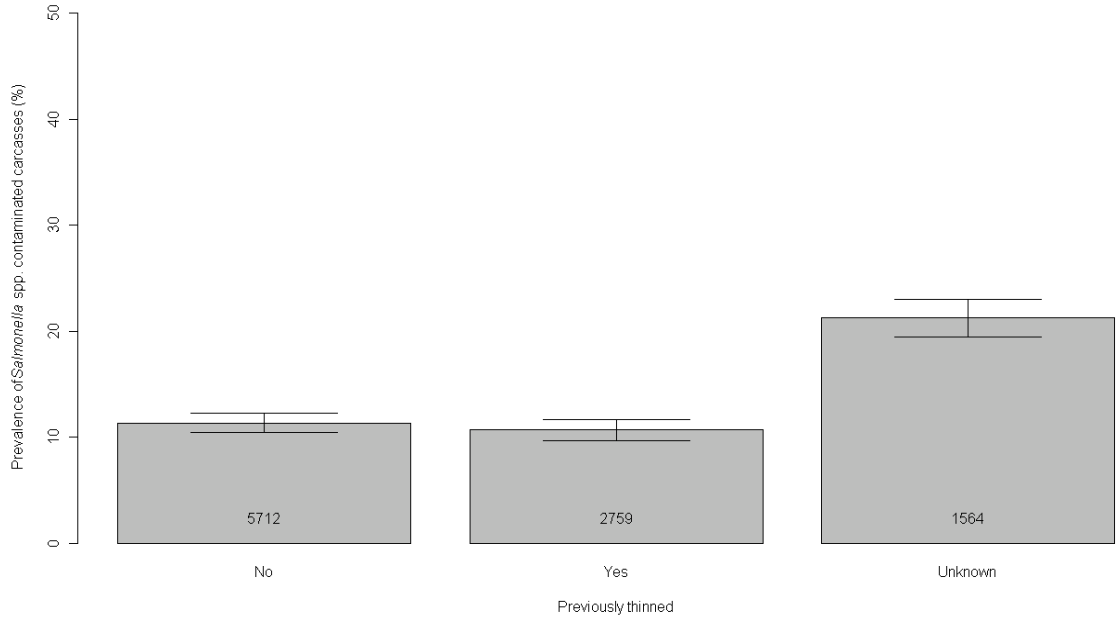

Figure 11: Prevalence of *Salmonella*-contaminated broiler carcasses by previous thinning in the EU [(a)] (based on 28 countries), 2008

Note: The numbers appearing within the shaded areas of the bars indicate the total number of sampled broiler carcasses for each category.

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

Table 11: Pearson's Chi-square to test for the independence between previous thinning in the flock and the *Salmonella* contamination result on the broiler carcass

	Chi-square statistic (*P*-value)
Salmonella spp.	150.18 (< 0.0001)

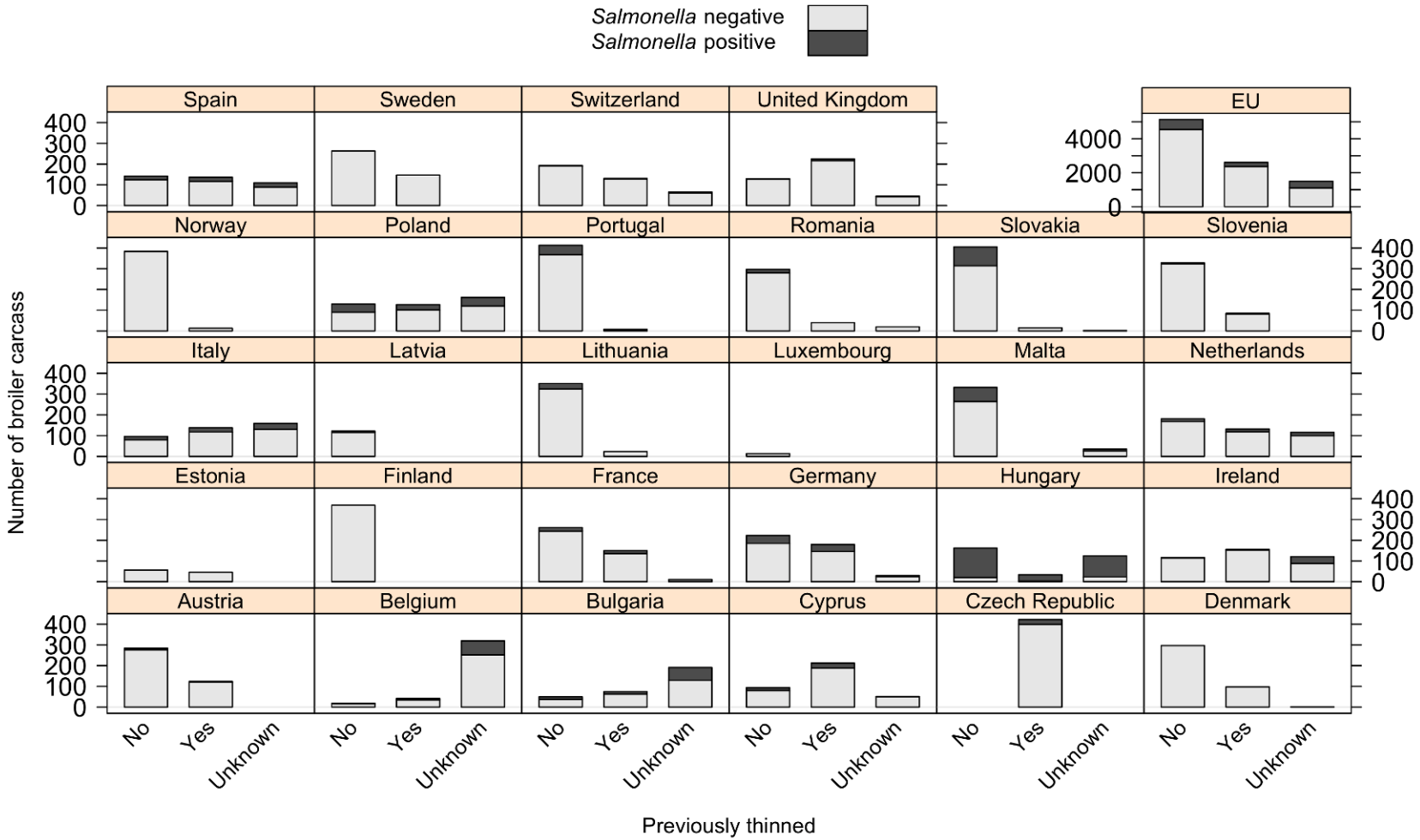

Figure 12: Distribution of *Salmonella*-contaminated broiler carcasses by previous thinning in the flock, by country and in the EU [(a)], 2008

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

Age of broilers

Table 12 reports some summary statistics for the age of the broilers (in days) for the sampled broiler carcasses in the EU. The *P*-value of the Cochran-Mantel-Haenszel Chi-square statistic (Table 13) is smaller than 0.05, thus there is a linear trend between the prevalence of *Salmonella*-contaminated broiler carcasses and the age of the broilers. Figure 13 displays the boxplot of the age of broilers by the *Salmonella* contamination result on the broiler carcasses showing that there is almost no difference between positive and negative samples. Figure 14 shows the boxplot of the age of broilers per country, while Figure 15 presents the boxplot of the same data, but distinguishing between *Salmonella*-positive and -negative samples.

Table 12: Summary statistics (minimum, maximum, mean and standard deviation) for the age of the broilers (in days) for the sampled broiler carcasses in the EU [(a)] (based on 28 countries), 2008

EU	Age of broilers				Total
	Min	**Max**	**Mean**	**Std**	**N**
	17	150	41.50	8.8	10,035

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

Table 13: Cochran-Mantel-Haenszel Chi-square test for the linear trend between the age of broilers and *Salmonella* contamination result on the broiler carcass

	Linear trend statistic (*P*-value)
Salmonella spp.	49.61 (< 0.0001)

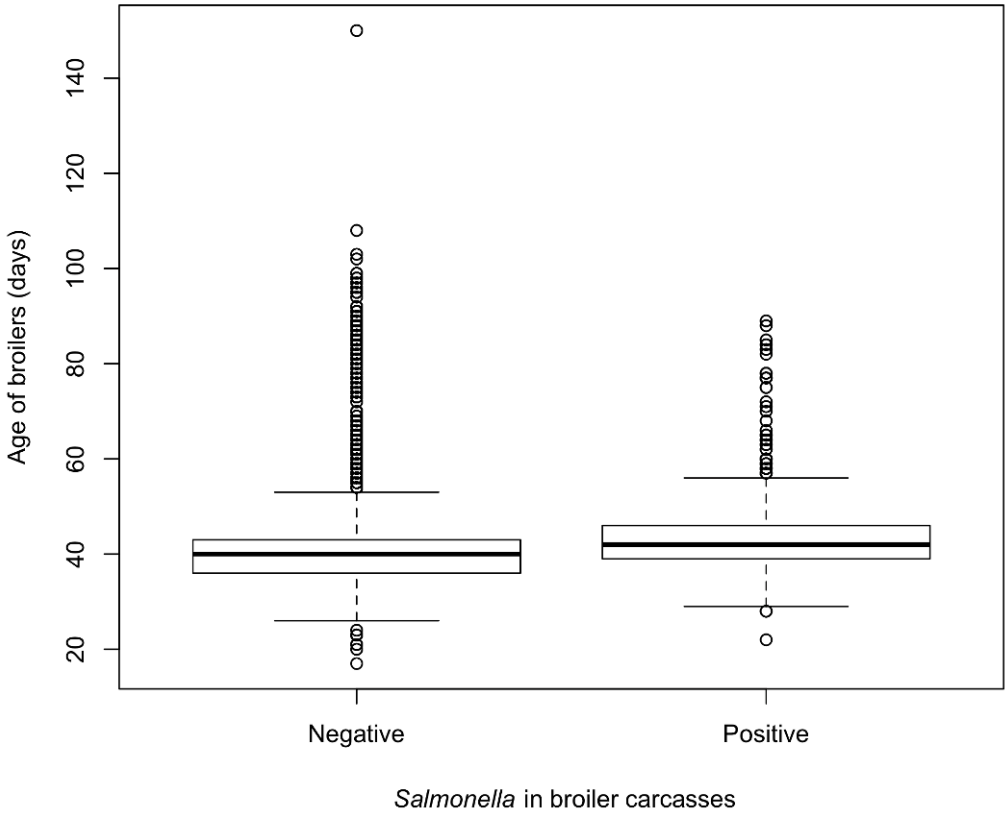

Figure 13: Boxplot of the age of broilers, by *Salmonella* contamination result on the broiler carcasses in the EU [(a)] (based on 28 countries), 2008

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

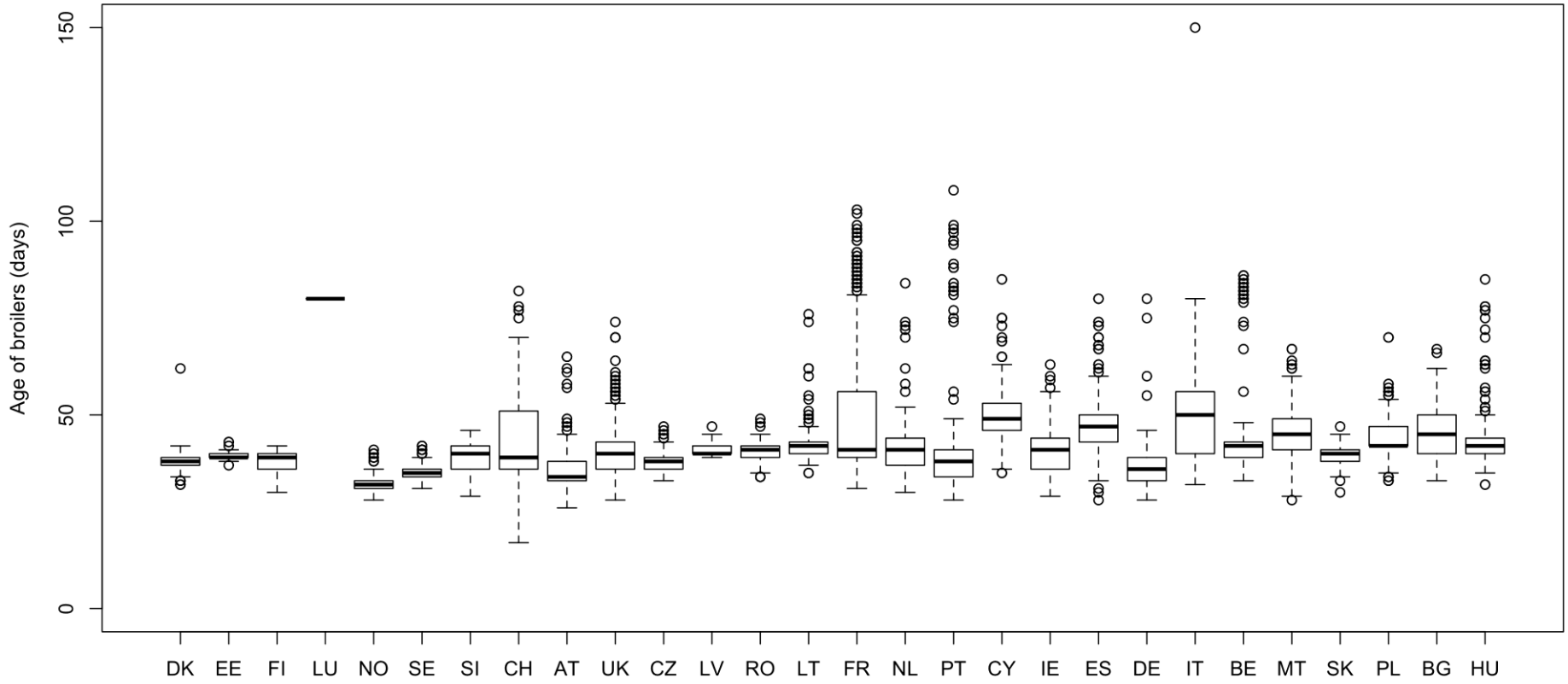

Figure 14: Boxplot of the age of broilers per country in the EU [(a)], 2008

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

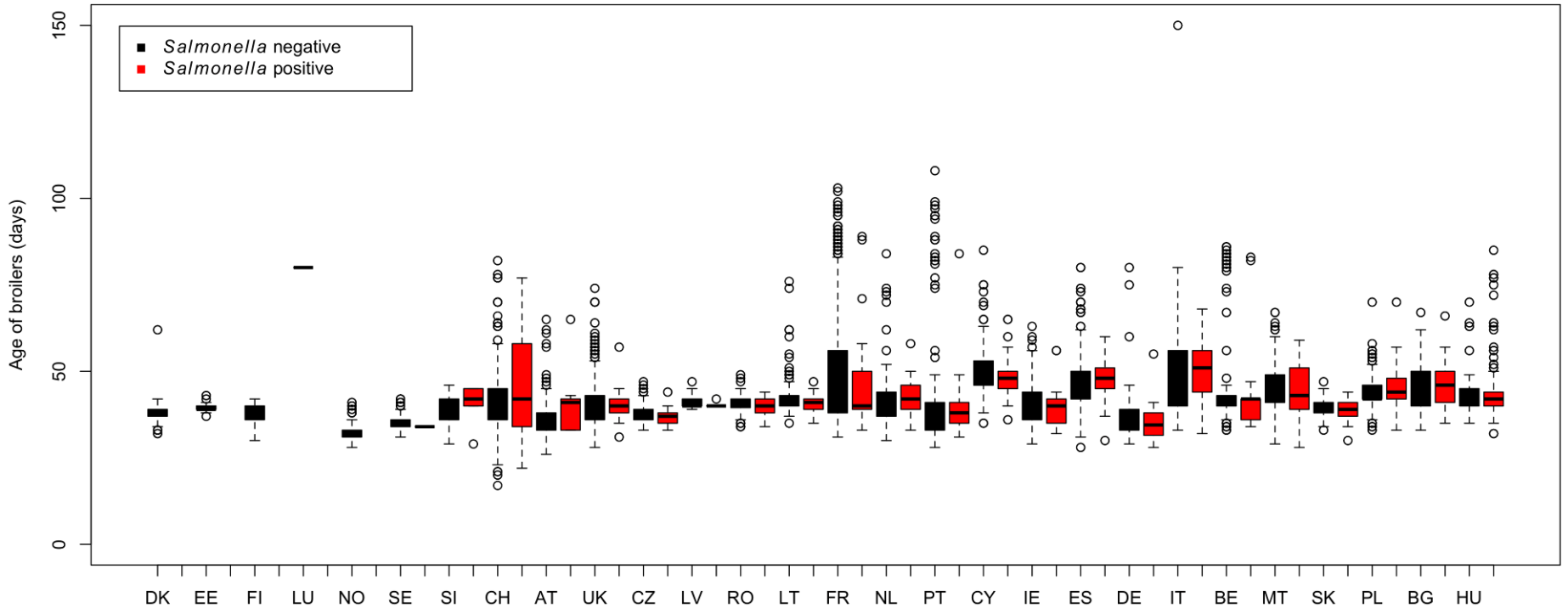

Figure 15: Boxplot of the age of broilers by countries (ordered by prevalence of *Salmonella* contamination on broiler carcasses) and by *Salmonella* contamination result on the broiler carcass in the EU [(a)], 2008

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

Quarter of sampling

Table 14 reports the numbers and percentages of broiler carcasses sampled in the EU per quarter of sampling. Figure 16 shows the barplot of the prevalence of *Salmonella*-contaminated broiler carcasses by quarter of sampling displaying a uniform prevalence over the four quarters. The *P*-value of the Pearson's Chi-square statistic (Table 15) is larger than 0.05, thus there is not any association between the prevalence of *Salmonella*-contaminated broiler carcasses and the quarter of sampling. A graphical display of the distribution of positive and negative broiler carcass samples collected by country and in the EU per quarter of sampling is presented in Figure 17.

Table 14: Number and percentage of sampled broiler carcasses by quarter of sampling in the EU [(a)] (based on 28 countries), 2008

Quarter of sampling	EU	
	N	%
January-March	2,118	*21.1*
April-June	2,574	*25.7*
July-September	2,598	*25.9*
October-December	2,745	*27.3*
Total	10,035	*100.0*

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

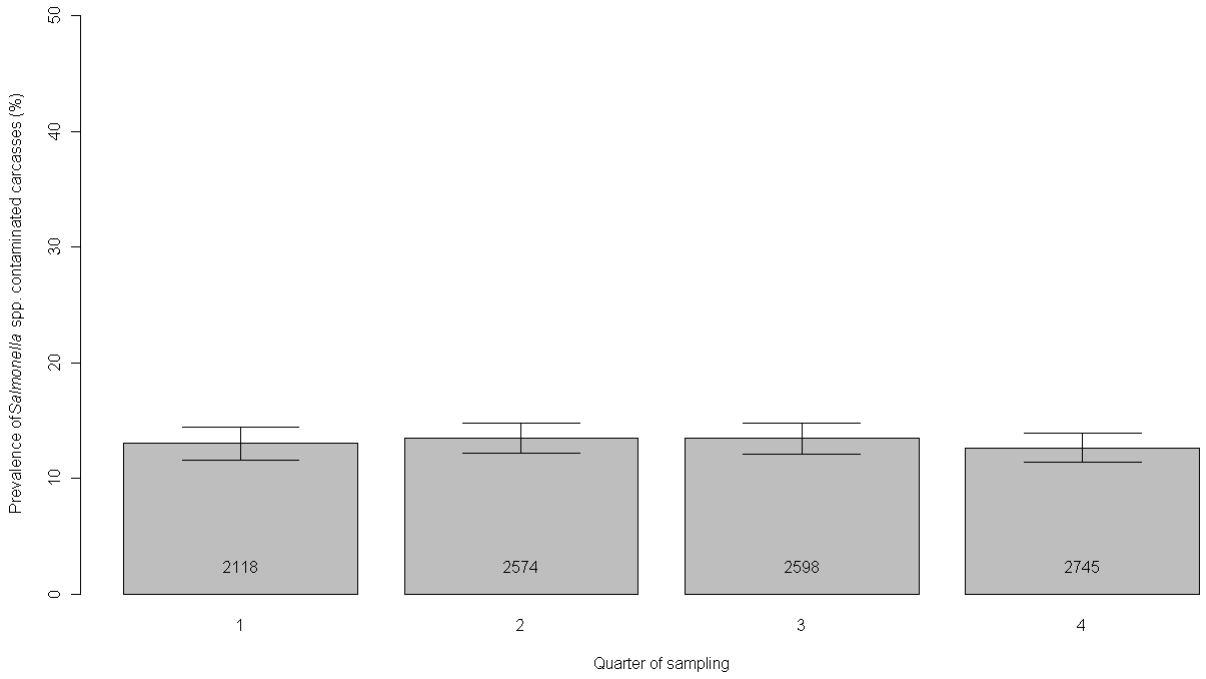

Figure 16: Prevalence of *Salmonella*-contaminated broiler carcasses by quarter of sampling in the EU [(a)] (based on 28 countries), 2008

Note: The numbers appearing within the shaded areas of the bars indicate the total number of sampled broiler carcasses for each category.

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

Table 15: Pearson's Chi-square test for association between quarter of sampling and *Salmonella* contamination result on the broiler carcass

	Chi-square statistic (*P*-value)
Salmonella spp.	1.06 (0.79)

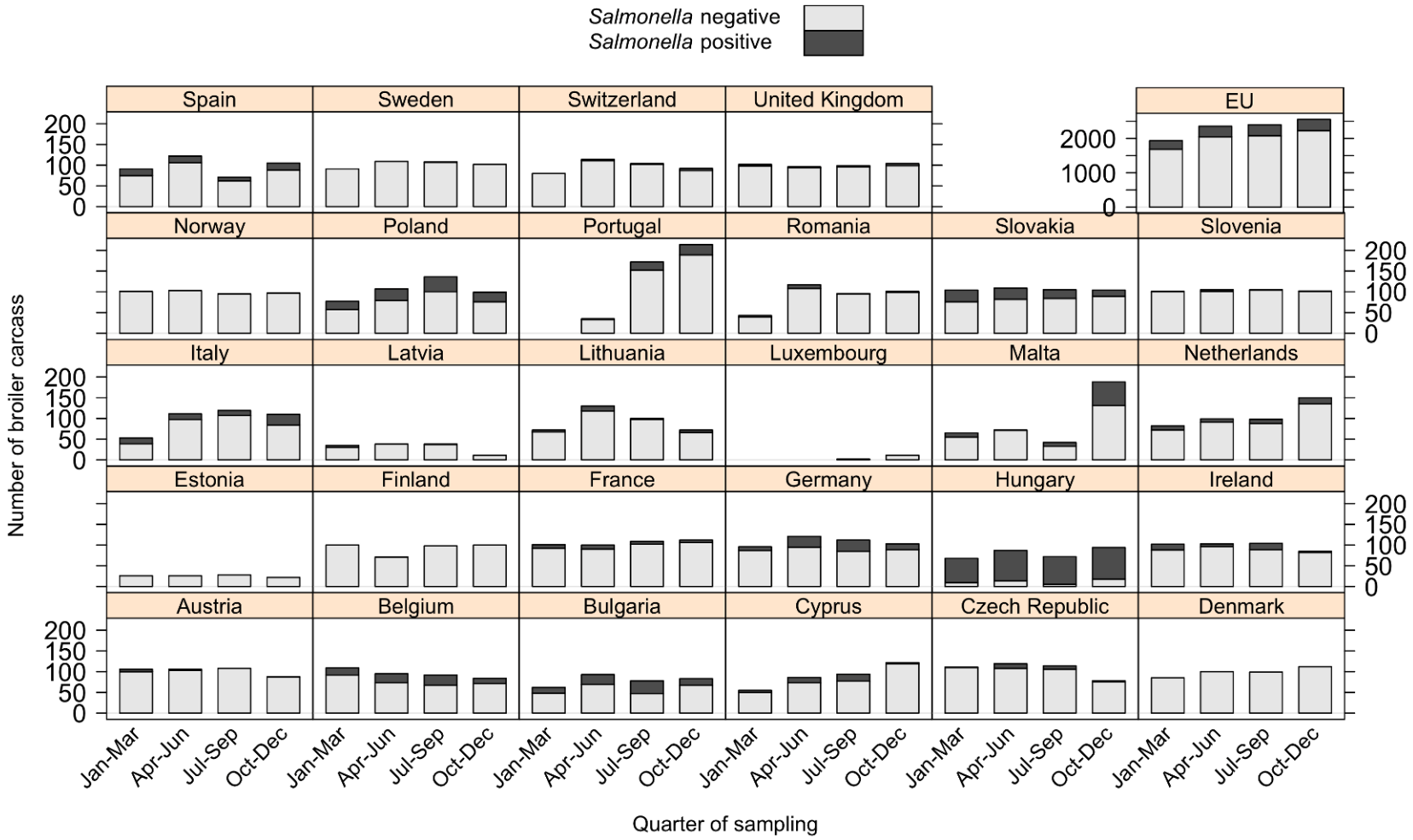

Figure 17: Distribution of *Salmonella*-contaminated and non-contaminated broiler carcasses by quarter of sampling, by country and in the EU [(a)], 2008

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

Time (hour) of sampling

Table 16 reports the numbers and percentages of broiler carcasses sampled in the EU per time (hour) of sampling. Figure 18 shows the distribution of positive and negative broiler carcasses sampled by country and in the EU per time (hour) of sampling. The *P*-value of the Cochran-Mantel-Haenszel Chi-square statistic (Table 17) is larger than 0.05, thus there is not any linear trend between the prevalence of *Salmonella*-contaminated broiler carcasses and the time (hour) of sampling. This test builds ad hoc Ridit scores from the scores previously defined.

Table 16: Number and percentage of sampled broiler carcasses by time (hour) of sampling in the EU [(a)] (based on 28 countries), 2008

Time (hour) of sampling	EU	
	N	%
< 9 am	4,517	*45.0*
9 - < 12am	3,342	*33.3*
12am – < 3pm	1,191	*11.9*
≥ 3pm	985	*9.8*
Total	10,035	*100.0*

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

Table 17: Cochran-Mantel-Haenszel Chi-square test for the linear trend between time (hour) of sampling and *Salmonella* contamination result on the broiler carcass

	Linear trend statistic (*P*-value)
Salmonella spp.	1.85 (0.17)

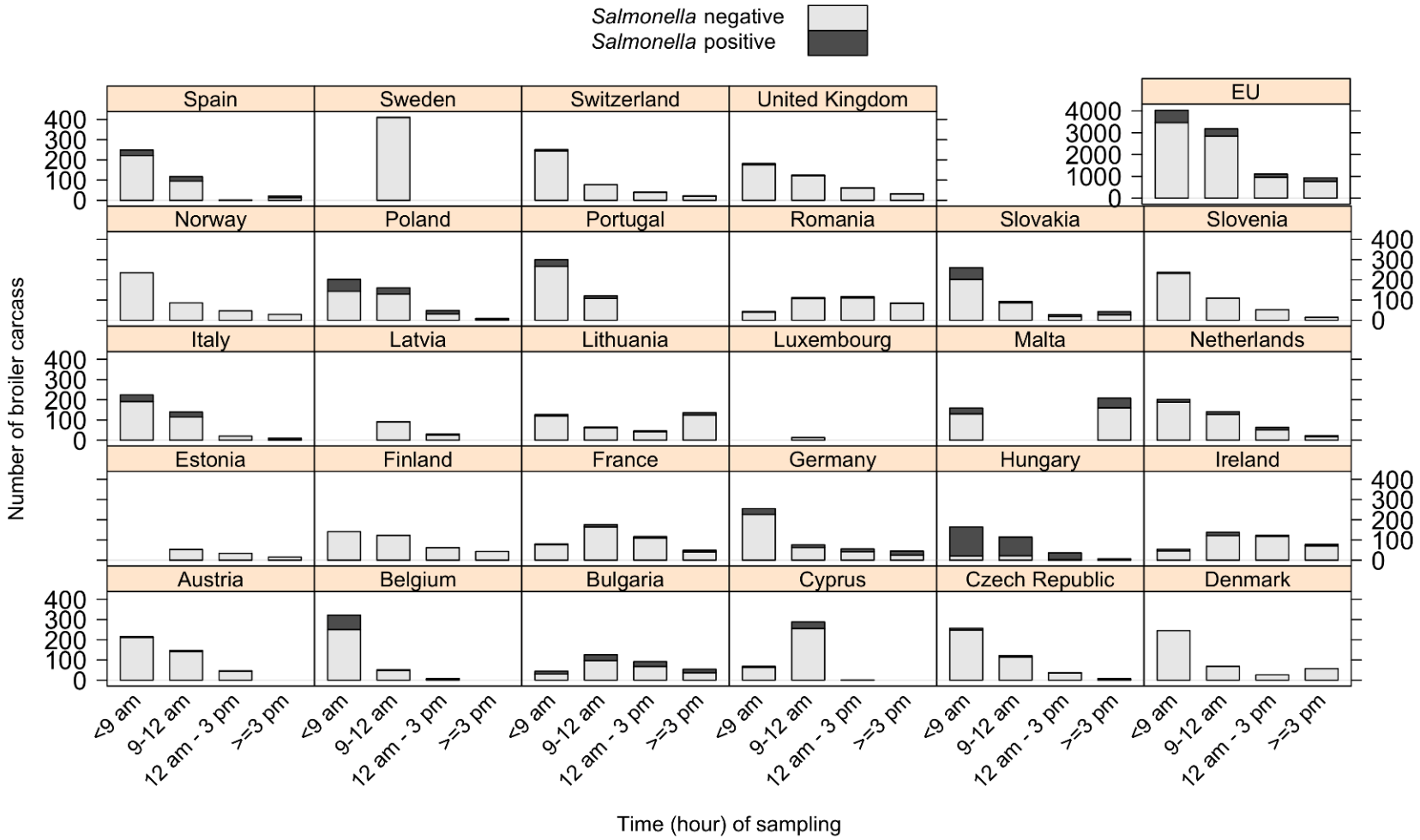

Figure 18: Distribution of *Salmonella*-contaminated carcasses by time (hour) of sampling, by country and in the EU [(a)], 2008

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

Time (hours) between sampling and testing

Table 18 reports the numbers and percentages of broiler carcasses sampled in the EU per time (hours) between sampling and testing. The *P*-value of the Cochran-Mantel-Haenszel Chi-square statistic (Table 19) is smaller than 0.05, thus there is a linear trend between the prevalence of *Salmonella*-contaminated broiler carcasses and the time (hours) between sampling and testing. This test builds ad hoc Ridit scores from the scores previously defined. Figure 19 displays the barplot of the prevalence of *Salmonella*-contaminated broiler carcasses by time (hours) between sampling and testing showing that the prevalence diminishes as the time (hours) between sampling and testing increases. Figure 20 shows the distribution of positive and negative sampled broiler carcasses by country and in the EU per time (hours) between sampling and testing.

Table 18: Number and percentage of sampled broiler carcasses according to time (hours) between sampling and testing in the EU [(a)] (based on 28 countries), 2008

Time (hours) between sampling and testing	EU	
	N	%
< 24 hours	3,190	*31.8*
24 hours - < 36 hours	4,141	*41.3*
36 hours - < 48 hours	1,136	*11.3*
48 hours - < 60 hours	995	*9.9*
60 hours - < 72 hours	261	*2.6*
72 hours - < 80 hours	312	*3.1*
Total	10,035	*100.0*

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

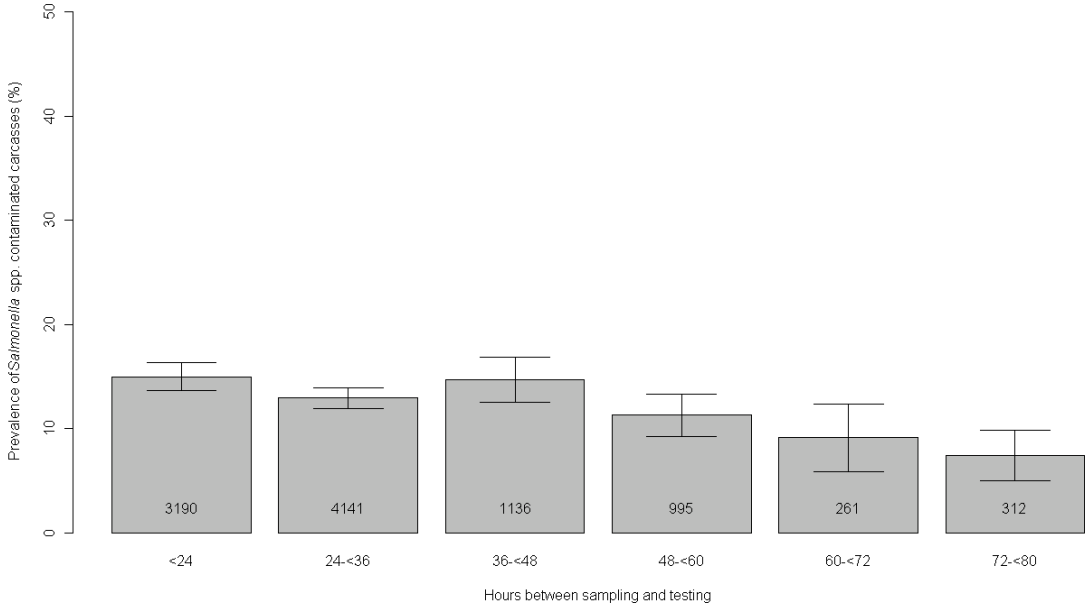

Figure 19: Prevalence of *Salmonella* prevalence in contaminated carcasses by hours between sampling and testing in the EU [(a)] (based on 28 countries), 2008

Note: The numbers appearing within the shaded areas of the bars indicate the total number of sampled broiler carcasses for each category.

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

Table 19: Cochran-Mantel-Haenszel Chi-square test for the linear trend between time (hours) between sampling and testing and the *Salmonella* contamination result on the broiler carcass

	Linear trend statistic (*P*-value)
Salmonella spp.	17.14 (< 0.0001)

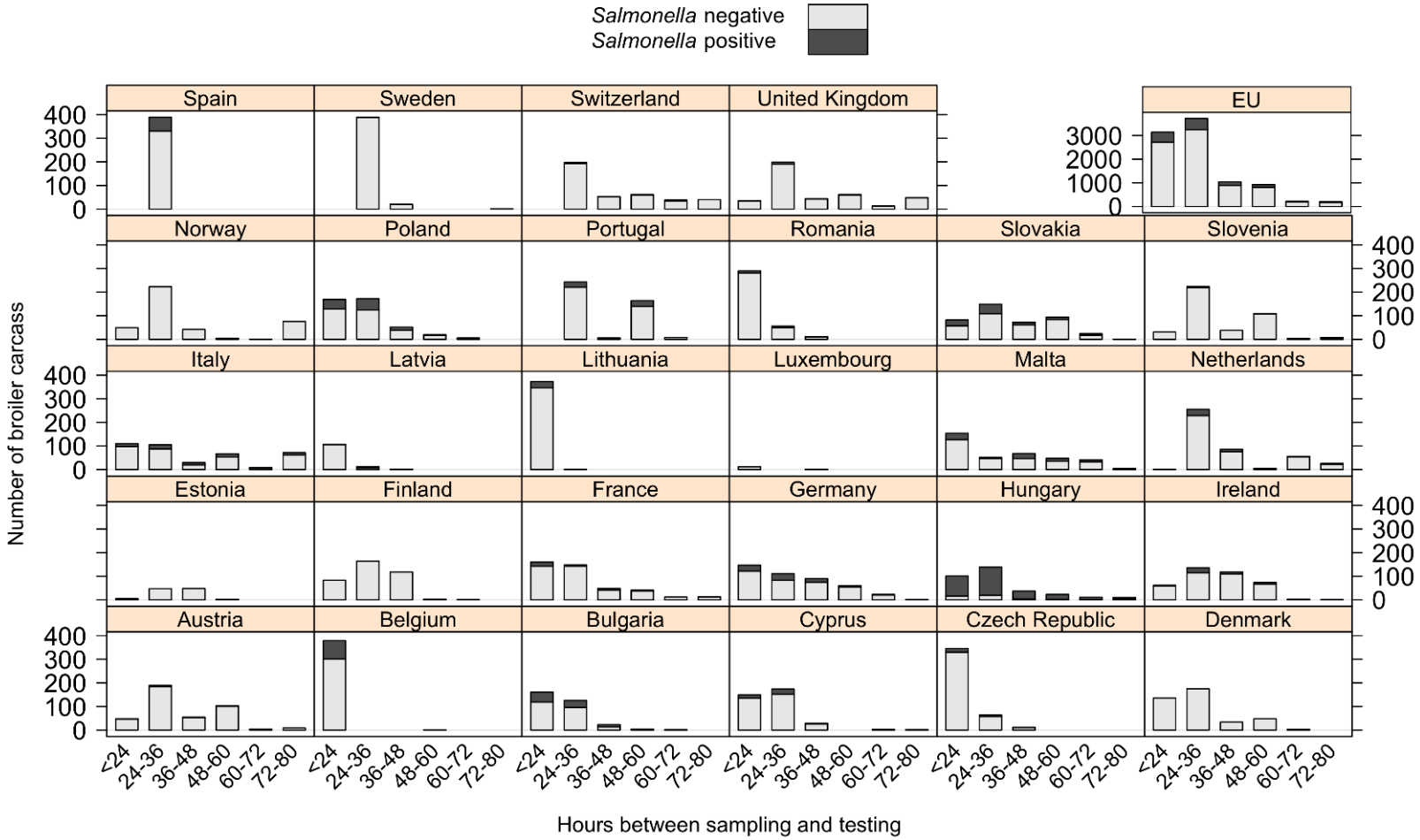

Figure 20: Distribution of *Salmonella* contaminated carcasses by hours between sampling and testing, by country and in the EU [(a)], 2008

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

Capacity of slaughterhouse

Table 20 reports the numbers and percentages of broiler carcasses sampled in the EU per capacity of the slaughterhouse. The *P*-value of the Cochran-Mantel-Haenszel Chi-square statistic (Table 21) is smaller than 0.05, thus there is a linear trend between the prevalence of *Salmonella*-contaminated broiler carcasses and the capacity of the slaughterhouse. This test builds ad hoc Ridit scores from the scores previously defined. Figure 21 displays the distribution of positive and negative sampled broiler carcasses by country and in the EU per capacity of the slaughterhouse.

Table 20: Number and percentage of the sampled broiler carcasses according to the capacity of the slaughterhouses in the EU [(a)], 2008

Capacity of slaughterhouse	EU	
	N	%
< 100,000	188	*1.9*
100,000-499,999	338	*3.4*
500,000-999,999	340	*3.4*
1,000,000-4,999,999	1,271	*12.7*
5,000,000-9,999,999	1,884	*18.8*
> 10,000,000	6,014	*59.9*
Total	10,035	*100.0*

(a) Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

Table 21: Cochran-Mantel-Haenszel Chi-square test for the linear trend between the capacity of slaughterhouses and *Salmonella* contamination result on the broiler carcass

	Linear trend statistic (*P*-value)
Salmonella spp.	92.23 (< 0.0001)

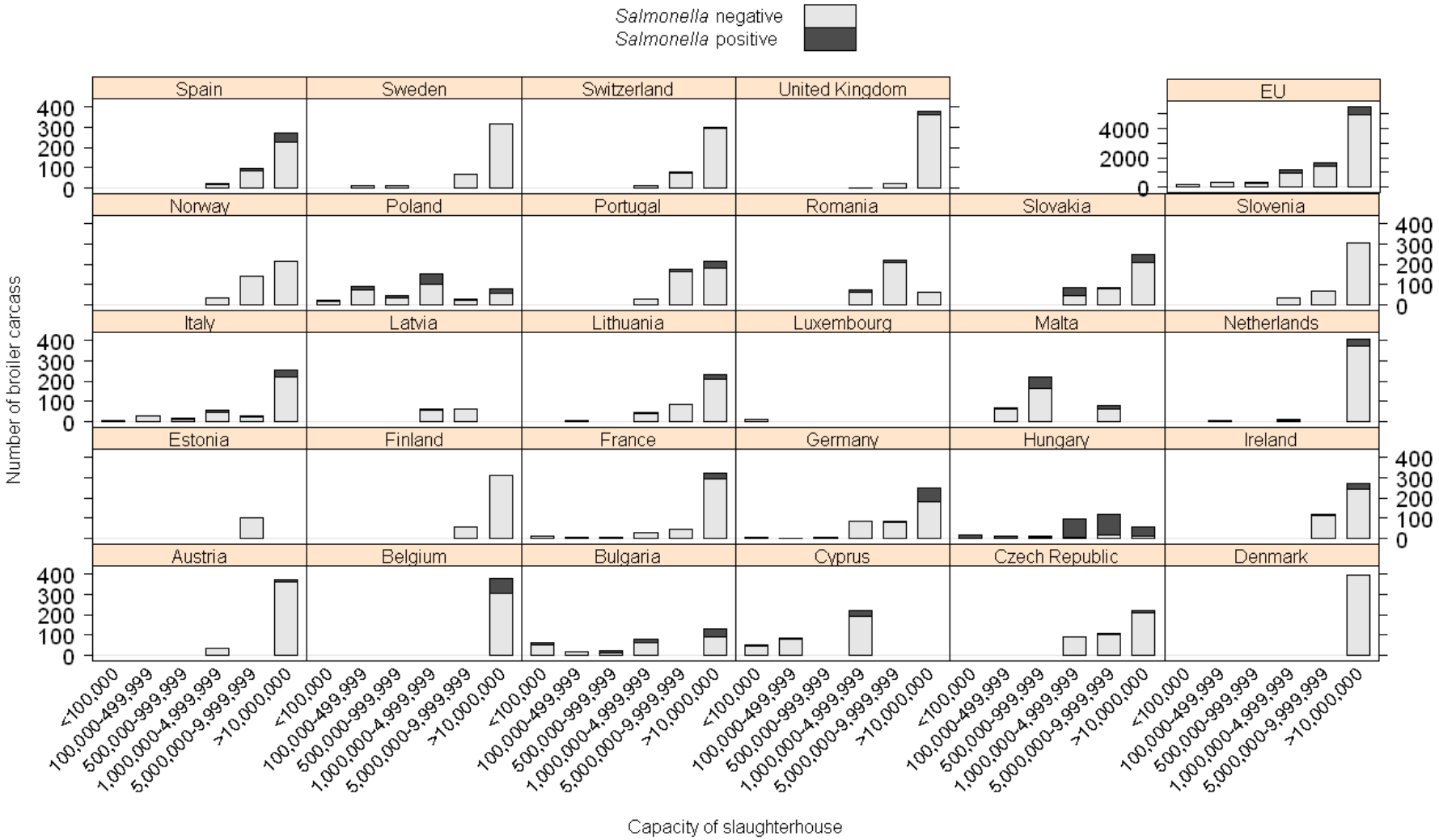

Figure 21: Distribution of *Salmonella*-contaminated carcasses by capacity of the slaughterhouses, by country and in the EU [(a)], 2008

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

Type of chilling of carcasses

For the purpose of this analysis, due to the small number of samples, we grouped the categories combining more than one chilling method in a fourth category called "mixed chilling".

Table 22 reports the numbers and percentages of broiler carcasses sampled in the EU per type of chilling of carcasses. Figure 22 displays the barplot of the prevalence of *Salmonella*-contaminated broiler carcasses by type of chilling showing that prevalence is similar between the individual types of chilling, apart from air chilling, which is the most used type of chilling and presents a lower prevalence. The *P*-value of the Pearson's Chi-square statistic (Table 23) is smaller than 0.05, thus there is an association between the prevalence of *Salmonella*-contaminated broiler carcasses and the type of chilling, because of the difference between air chilling and the other types. A graphical display of the distribution of positive and negative broiler carcass samples collected by country and in the EU per type of chilling is presented in Figure 23.

Table 22: Number and percentage of sampled broiler carcasses by type of carcass chilling the EU [(a)] (based on 28 countries), 2008

Type of chilling of carcasses	EU	
	N	%
Air	7,220	*72.0*
Immersion	780	*7.8*
Spray	1,558	*15.5*
Mixed	477	*4.8*
Total	10,035	*100.0*

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

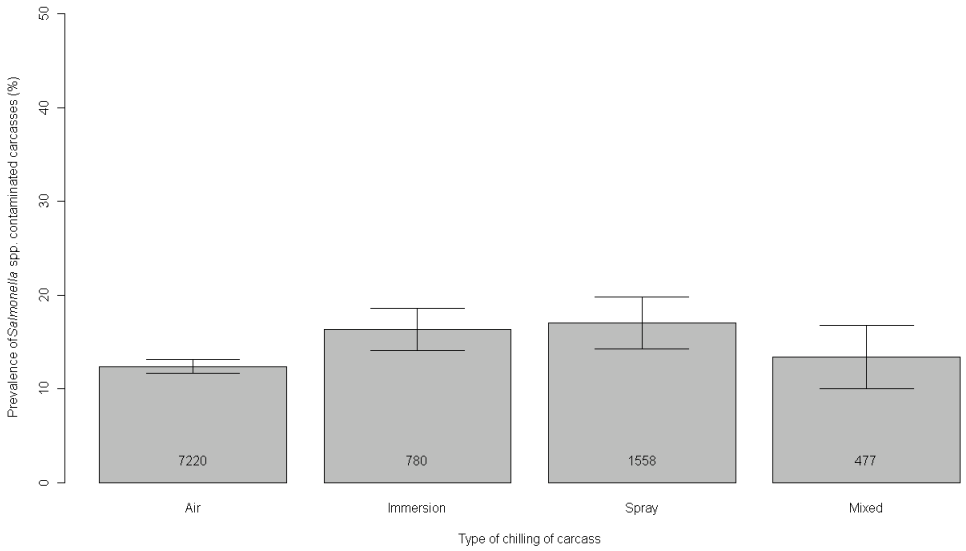

Figure 22: Prevalence of *Salmonella*-contaminated broiler carcasses by type of carcass chilling in the EU [(a)] (based on 28 countries), 2008

Note: The numbers appearing within the shaded areas of the bars indicate the total number of sampled broiler carcasses for each category.

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

Table 23: Pearson's Chi-square to test for the independence between the type of carcass chilling and the *Salmonella* contamination result on broiler carcasses

	Chi-square statistic (*P*-value)
Salmonella spp.	22.51 (< 0.0001)

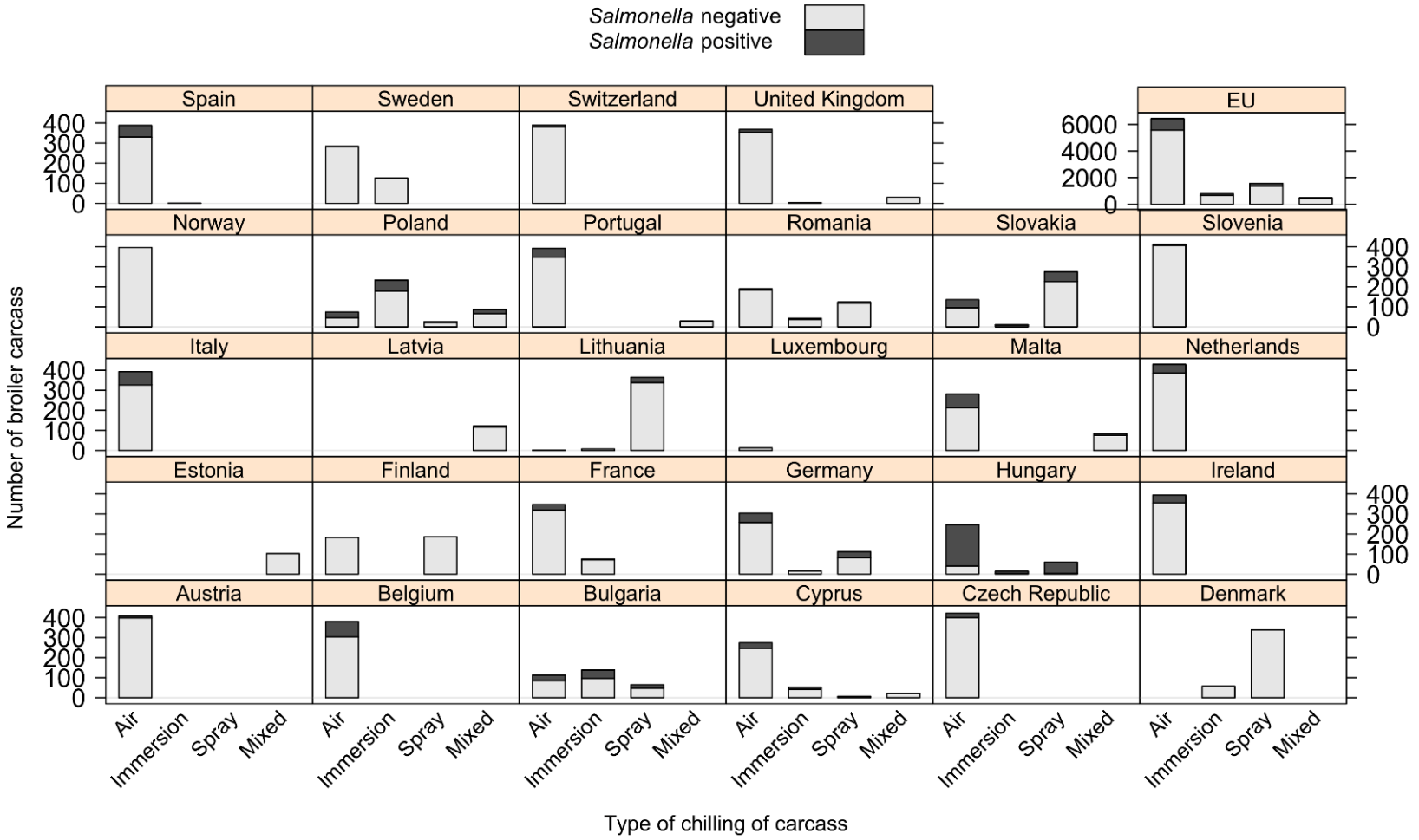

Figure 23: Distribution of *Salmonella*-contaminated broiler carcasses by type of carcass chilling, by country and in the EU [(a)], 2008

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

Campylobacter contamination result on broiler carcasses

Table 24 reports the numbers and percentages of broiler carcasses sampled in the EU per *Campylobacter* contamination result on the broiler carcasses. Figure 24 displays the barplot of the prevalence of *Salmonella*-contaminated broiler carcasses by *Campylobacter* contamination result on the broiler carcasses showing that there is no significant difference in prevalence whatever the *Campylobacter* contamination result on the broiler carcasses. The *P*-value of the Pearson's Chi-square statistic (Table 25) is higher than 0.05, thus there is no association between the prevalence of *Salmonella*-contaminated broiler carcasses and the *Campylobacter* contamination result on broiler carcasses. Figure 25 shows a graphical display of the distribution of positive and negative broiler carcass samples collected by country and in the EU per *Campylobacter* contamination result on broiler carcasses.

Table 24: Number and percentage of sampled broiler carcasses by *Campylobacter* contamination result on broiler carcasses in the EU [(a)] (based on 28 countries), 2008

***Campylobacter* result on broiler carcasses**	**EU**	
	N	**%**
Negative	4,130	*41.2*
Positive	5,869	*58.5*
Total [(b)]	10,035	*100.0*

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.
(b): For 36 *Salmonella* samples corresponding *Campylobacter* results were missing.

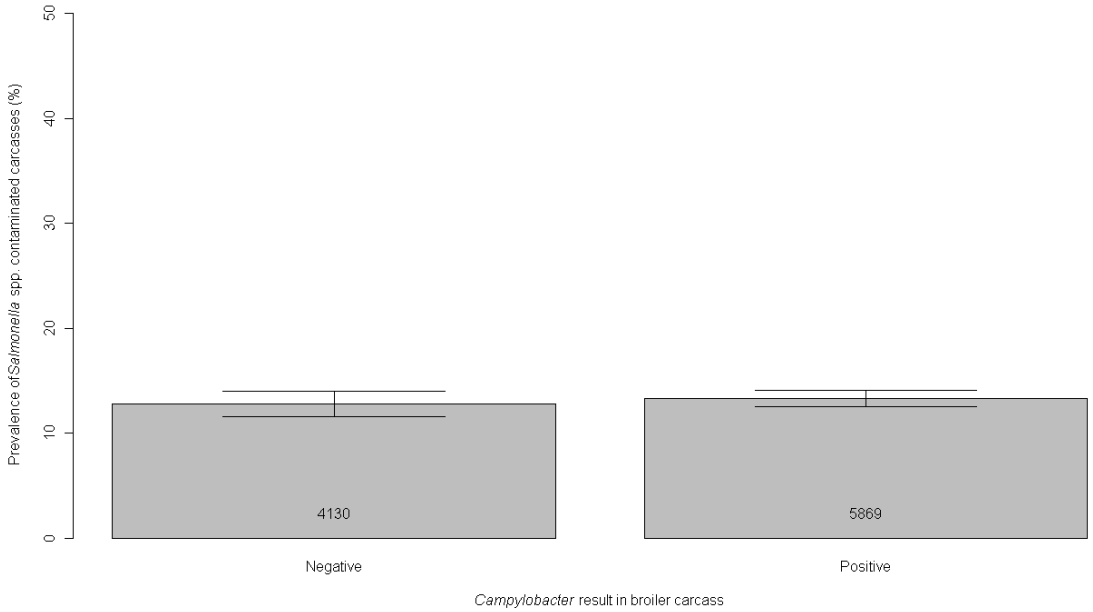

Figure 24: Prevalence of *Salmonella*-contaminated broiler carcasses by *Campylobacter* contamination result on the broiler carcasses in the EU [(a)] (based on 28 countries), 2008

Note: The numbers appearing within the shaded areas of the bars indicate the total number of sampled broiler carcasses for each category.
(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis. For 36 samples the results were missing.

Table 25: Pearson's Chi-square to test for the independence between the *Campylobacter* contamination result on broiler carcasses and the *Salmonella* contamination result on broiler carcasses

	Chi-square statistic (*P*-value)
Salmonella spp.	0.47 (0.49)

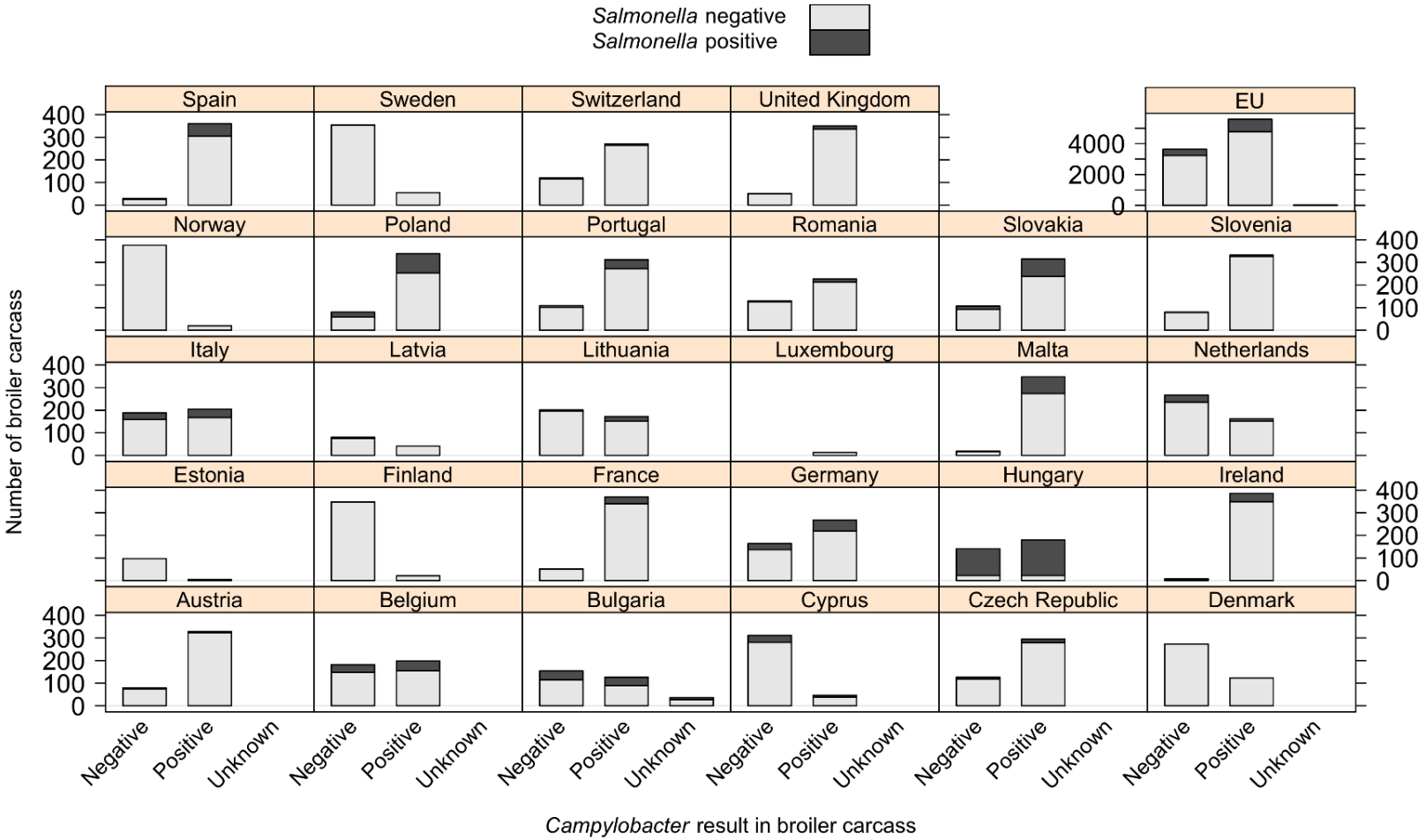

Figure 25: Distribution of *Salmonella*-contaminated broiler carcasses by *Campylobacter* contamination result on broiler carcasses, by country and in the EU [(a)], 2008

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

C. FINAL MODEL FOR THE *SALMONELLA* CONTAMINATION RESULT ON THE BROILER CARCASS: VARIANCE INFLATION FACTOR VALUES

Table 26: Variance Inflation Factor values for factors potentially related to *Salmonella*-contaminated broiler carcasses

Risk Factor	VIF
Flock production type	1.26
Previous thinning in the flock	1.20
Age of broilers	1.47
Quarter of sampling	1.03
Time (hour) of sampling	1.30
Hours between sampling and testing	1.08
Capacity of slaughterhouse	1.28
Type of chilling of carcasses	1.37
Campylobacter contamination result on the broiler carcass	1.37

Note: Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

D. FULL MODEL FOR *SALMONELLA*-CONTAMINATED BROILER CARCASSES IN COUNTRIES WITH PREVALENCE ABOVE THE EU MEDIAN PREVALENCE AND COUNTRIES WITH PREVALENCE BELOW THE EU[14] MEDIAN PREVALENCE

Table 27: Comparison of the full models for the *Salmonella* result on broiler carcasses between countries with prevalence below the EU median prevalence and above the EU median prevalence: odds ratio and 95% confidence intervals for the risk factors and *P*-value of the type III text for fixed effects

	Variable	Levels	OR	Lower	Upper	*P*-value
Countries with prevalence below the EU[(a)] median prevalence	Flock production type Reference category: *Conventional*	*Organic* *Standard*	1.305 4.263	0.073 0.427	23.378 42.557	0.465
	Previous thinning in the flock Reference category: *No*	*Unknown* *Yes*	2.308 0.778	0.375 0.244	14.227 2.487	0.561
	Age of broilers		0.631	0.283	1.409	0.261
	Quarter of sampling Reference category: *I*	*IV* *III* *II*	0.994 0.787 1.045	0.331 0.198 0.316	2.984 3.126 3.457	0.900
	Time (hour) of sampling Reference category: *< 9am*	*≥ 3pm* *12am - < 3pm* *9-< 12am*	2.41 1.794 1.058	0.498 0.638 0.373	11.664 5.044 3.003	0.568
	Capacity of slaughterhouse		0.358	0.058	2.203	0.268
	Type of chilling of carcass Reference category: *Air*	*Mixed* *Spray* *Immersion*	0.015 2.135 0.849	0.002 0.445 0.13	0.114 10.235 5.55	0.001
	Campylobacter contamination result on the broiler carcass Reference category: *Negative*	*Positive*	1.242	0.743	2.075	0.408
Countries with prevalence above the EU[(a)] median prevalence	Flock production type Reference category: *Conventional*	*Unknown* *Organic* *Standard*	1.051 1.233 0.765	0.497 0.322 0.377	2.226 4.73 1.551	0.841
	Previous thinning in the flock Reference category: *No*	*Unknown* *Yes*	1.296 1.012	0.891 0.715	1.884 1.434	0.331
	Age of broilers		1.163	0.953	1.419	0.138
	Quarter of sampling Reference category: *I*	*IV* *III* *II*	0.835 0.894 0.975	0.597 0.606 0.688	1.169 1.321 1.382	0.700
	Time (hour) of sampling Reference category: *< 9am*	*≥ 3pm* *12am - < 3pm* *9-< 12am*	3.015 1.79 0.994	1.868 1.138 0.699	4.865 2.814 1.414	< 0.0001
	Capacity of slaughterhouse		2.324	1.464	3.69	0.0004
	Type of chilling of carcass Reference category: *Air*	*Mixed* *Spray* *Immersion*	0.818 0.481 0.918	0.283 0.158 0.411	2.366 1.464 2.053	0.607
	Campylobacter contamination result on the broiler carcass Reference category: *Negative*	*Positive*	1.097	0.831	1.449	0.512

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis. The factor 'time in hours between sampling and testing' was only used to adjust the model.

[14] Two non-MSs, Norway and Switzerland, were included in the overall EU level dataset.

E. ANALYSIS OF THE VARIANCE COMPONENTS

Table 28 shows the estimate of the variance of the slaughterhouse-specific random intercepts. The variance of the random intercepts (effect of slaughterhouse) is significantly different from zero. The Wald test statistic is 50.9 (*P*-value <0.001) and was calculated using a 50:50 mixture of Chi-square distributions with 0 and 1 degrees of freedom, given that the value under the null hypothesis lies on the border of the parameter space (Molenberghs and Verbeke, 2005). Since the standard error is much smaller than the estimate, the null hypothesis that the variance is zero can be rejected and this is confirmed by the likelihood ratio test for the need of a random intercept in the model: with a test value of 867, it can be concluded that there is a significant difference in terms of goodness-of-fit compared to a fixed effects model. The fact that the variance of the random effects is moderate and significantly positive indicates that there is heterogeneity among the slaughterhouses: this means that there are some slaughterhouses within countries that have a more positive intercept, giving a higher prevalence, while the slaughterhouses within countries with a more negative intercept give a lower prevalence.

Table 28: Final random effect logistic model for factors associated with *Salmonella*-contaminated broiler carcasses: estimate and standard error of the variance of the slaughterhouse-specific random intercepts and likelihood ratio test

	Estimate	**Std Error**	***P*-value**
Variance of the slaughterhouse-specific random intercepts	2.824	0.396	<0.001
Likelihood ratio test for the need of a random intercept	866.99	-	<0.0001

The intra-cluster correlation associated with the slaughterhouse-specific random effects can be estimated as follows:

$$ICC = \frac{\sigma_b^2}{\sigma_b^2 + \pi^2/3},$$

and results in *ICC* = 0.46. This implies that 46% of the variability not explained by the covariates, might be explained by differences between slaughterhouses.

Table 29 shows the estimates of the variance of the slaughterhouse-specific random intercepts for the model with countries below the EU median prevalence and the countries above it. The variance of the random intercepts (effect of slaughterhouses) is significantly different from zero in both cases. The Wald test statistics are equal to 7.2 (*P*-value = 0.004), for the countries below the median, and 39.7 (*P*-value <0.0001), for the countries above the median. The *P*-values are calculated using a 50:50 mixture of Chi-square distributions with 0 and 1 degrees of freedom, because the values under the null hypothesis lie on the border of the parameter space (Molenberghs and Verbeke, 2005). The fact that the variance of the random effects is moderate and significantly positive indicates that there is heterogeneity among the slaughterhouses: this means that there are some slaughterhouses within countries that have a more positive intercept, giving a higher prevalence, while the slaughterhouses within countries with a more negative intercept give a lower prevalence.

Table 29: Model for the *Salmonella* result on broiler carcasses - estimate and standard error of the variance of the random intercepts for the slaughterhouses

	Estimate	**Standard Error**	***P*-value**
Below the EU[15] median prevalence	1.301	0.485	0.004
Above the EU median prevalence	3.145	0.499	<0.0001

[15] Two non-MSs, Norway and Switzerland, were included in the overall EU level dataset.

F. ANALYSIS OF THE *SALMONELLA* SEROVARS DISTRIBUTION

Table 30: Frequency distribution of the top 20 *Salmonella* serovars from contaminated broiler carcasses in the EU [(a)], 2008.

Serovar	Carcasses (N=1,225[(b)])		No of countries
	N	%	
S. Infantis	358	*29.2*	15
S. Enteritidis	166	*13.6*	14
S. Kentucky	76	*6.2*	6
S. Typhimurium	54	*4.4*	10
S. Bredeney	53	*4.3*	7
S. Virchow	50	*4.1*	6
S. Hadar	47	*3.8*	9
S. Paratyphi B var. Java	46	*3.8*	3
S. Agona	37	*3.0*	10
S. Indiana	35	*2.9*	6
S. Montevideo	32	*2.6*	7
S. Mbandaka	30	*2.4*	10
S. Blockley	22	*1.8*	5
S. 4,12:d:-	21	*1.7*	1
S. Thompson	21	*1.7*	5
S. 4,[5],12:i:-	15	*1.2*	4
S. Livingstone	12	*1.0*	4
S. 6,7:-:-	11	*0.9*	2
S. Ohio	11	*0.9*	5
S. Derby	10	*0.8*	3
Others	95	*7.7*	-
Salmonella untypeable	55	*4.5*	6

(a): The total number of broiler carcasses includes all carcasses where at least one *Salmonella* serovar was isolated.
(b): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

Table 31: Frequency of the top 20 *Salmonella* serovars found on contaminated broiler carcasses in the EU[(a)], 2008

Country	AT	BE	BG	CY	CZ	DE	DK	EE	ES	FI	FR	HU	IE	IT	LT	LU	LV	MT	NL	PL	PT	RO	SI	SK	SE	UK	EU Total	CH	NO	Total
No of samples in study	**408**	**380**	**316**	**357**	**422**	**432**	**396**	**102**	**389**	**369**	**422**	**321**	**394**	**393**	**374**	**13**	**122**	**367**	**429**	**419**	**421**	**357**	**410**	**413**	**422**	**401**	**9,249**	**390**	**396**	**10,035**
Serovar																														
S. Infantis	1	7	13	3	1	6	0	0	2	0	0	269	0	1	0	0	0	3	3	26	0	0	4	15	0	0	354	4	0	358
S. Enteritidis	2	0	18	0	4	0	0	0	21	0	1	13	0	1	1	0	6	0	0	30	38	2	2	27	0	0	166	0	0	166
S. Kentucky	1	0	0	0	2	0	0	0	0	0	0	0	39	0	0	0	0	15	0	0	0	0	0	14	0	5	76	0	0	76
S. Typhimurium	1	11	1	0	0	20	0	0	5	0	0	1	0	0	0	0	0	0	1	10	0	1	0	0	0	0	51	3	0	54
S. Bredeney	0	0	0	11	0	8	0	0	1	0	0	0	0	1	0	0	0	28	0	0	0	3	0	0	0	1	53	0	0	53
S. Virchow	0	18	5	0	0	0	0	0	7	0	0	0	0	1	0	0	0	0	0	11	0	8	0	0	0	0	50	0	0	50
S. Hadar	0	1	0	9	0	1	0	0	6	0	1	0	0	18	0	0	0	2	1	8	0	0	0	0	0	0	47	0	0	47
S. Paratyphi B var. Java	0	7	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	30	0	0	0	0	0	0	0	46	0	0	46
S. Agona	0	5	0	0	12	0	0	0	0	0	2	0	0	0	3	0	0	0	1	4	0	0	0	7	1	1	36	1	0	37
S. Indiana	0	1	0	0	0	4	0	0	0	0	12	2	0	0	0	0	0	0	1	1	0	0	0	14	0	0	35	0	0	35
S. Montevideo	4	2	21	0	1	0	0	0	0	0	2	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	32	0	0	32
S. Mbandaka	0	0	0	0	0	1	0	0	4	0	1	0	0	3	1	0	0	0	1	10	6	0	0	1	0	2	30	0	0	30
S. Blockley	0	3	0	9	0	4	0	0	5	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	22	0	0	22
S. 4,12:d:-	0	0	0	0	0	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	0	0	21
S. 4,[5],12:i:-	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	12	1	0	0	0	0	0	0	0	14	1	0	15
S. Thompson	0	0	3	0	0	0	0	0	1	0	1	4	0	12	0	0	0	0	0	0	0	0	0	0	0	0	21	0	0	21
S. Livingstone	0	3	0	0	0	0	0	0	0	0	1	0	0	7	0	0	0	0	0	0	0	0	0	0	0	1	12	0	0	12
S. 6,7:-:-	0	0	10	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	11	0	0	11
S. Ohio	0	0	0	0	2	4	0	0	1	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	1	11	0	0	11
S. Derby	0	0	0	1	0	0	0	0	0	0	4	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	10
Others	1	4	14	5	1	1	0	0	5	0	11	0	0	2	5	0	0	7	0	6	2	3	1	13	0	3	94	1	0	95
Salmonella untypeable	0	15	0	0	0	0	0	0	0	0	0	0	0	13	15	0	0	10	1	0	1	0	0	0	0	0	55	0	0	55

(a): Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

G. SPATIAL DISTRIBUTION OF *SALMONELLA* SEROVARS ON *SALMONELLA*-CONTAMINATED BROILER CARCASSES

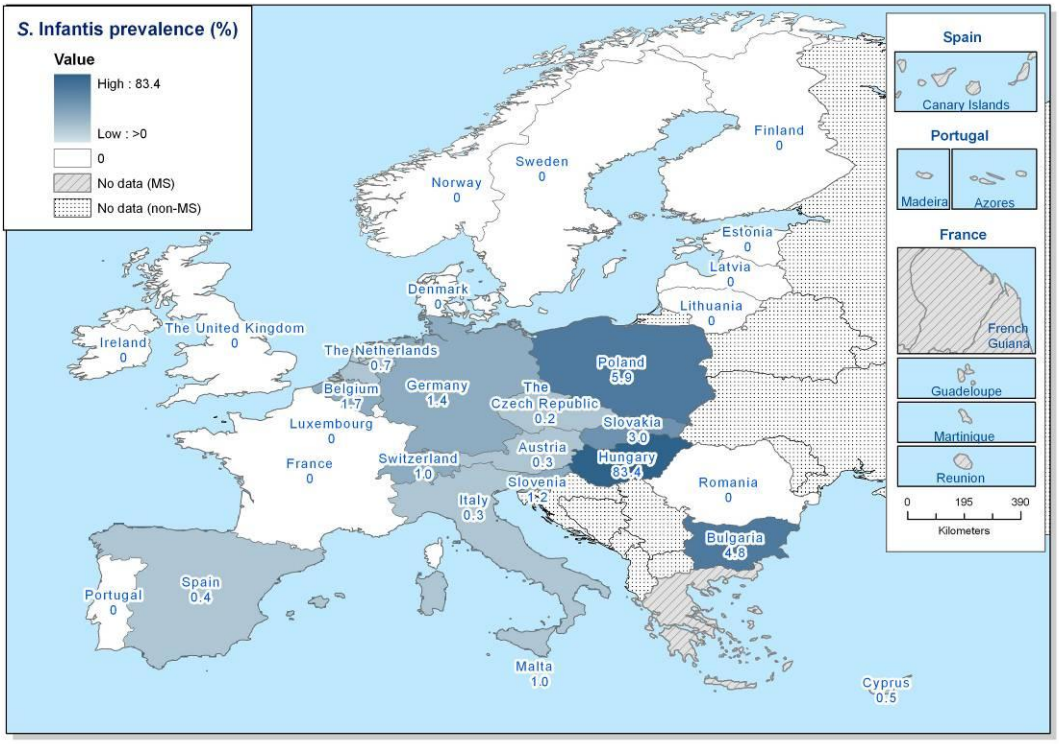

Figure 26: Distribution of the estimated prevalence of *S.* Infantis in countries participating in the broiler carcass baseline survey, 2008

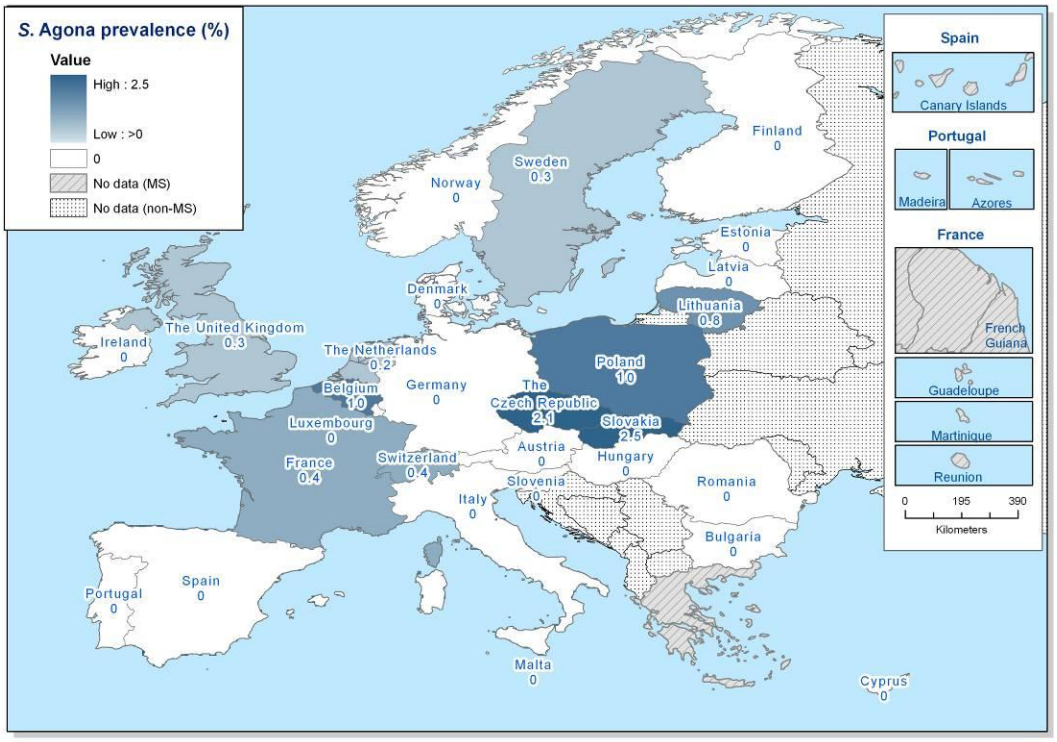

Figure 27: Distribution of the estimated prevalence of *S.* Agona in countries participating in the broiler carcass baseline survey, 2008

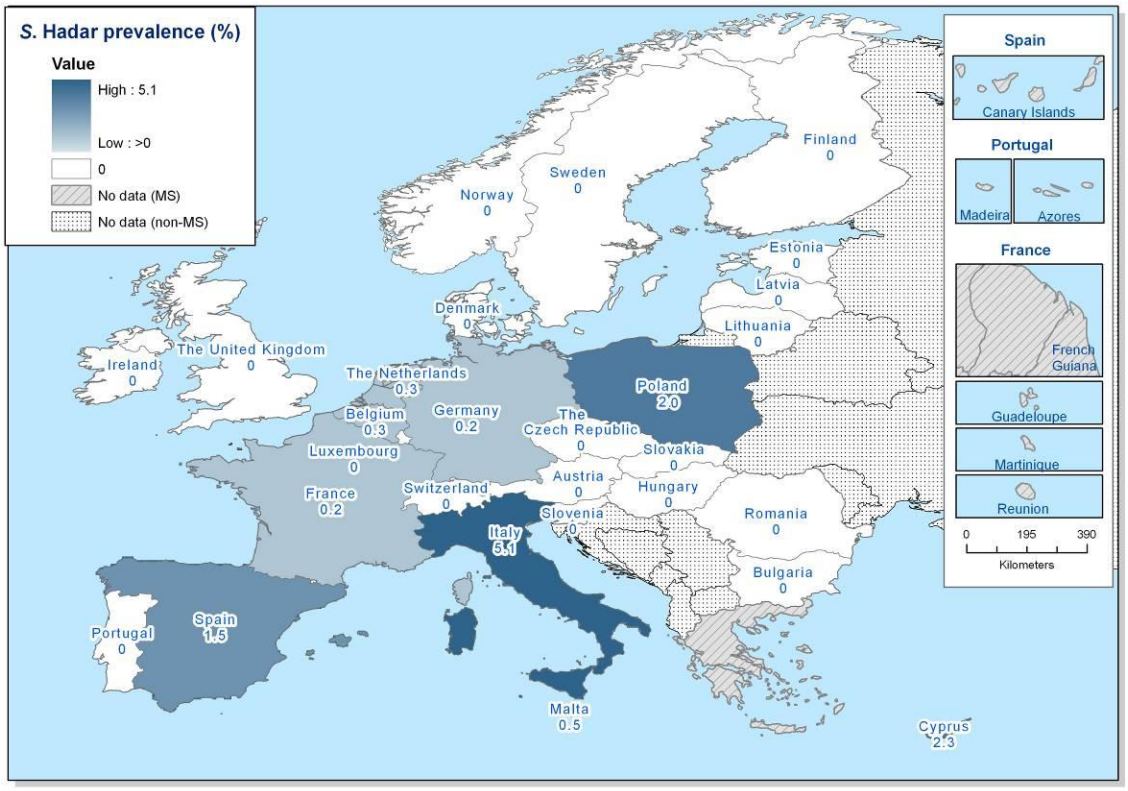

Figure 28: Distribution of the estimated prevalence of *S.* Hadar in countries participating in the broiler carcass baseline survey, 2008

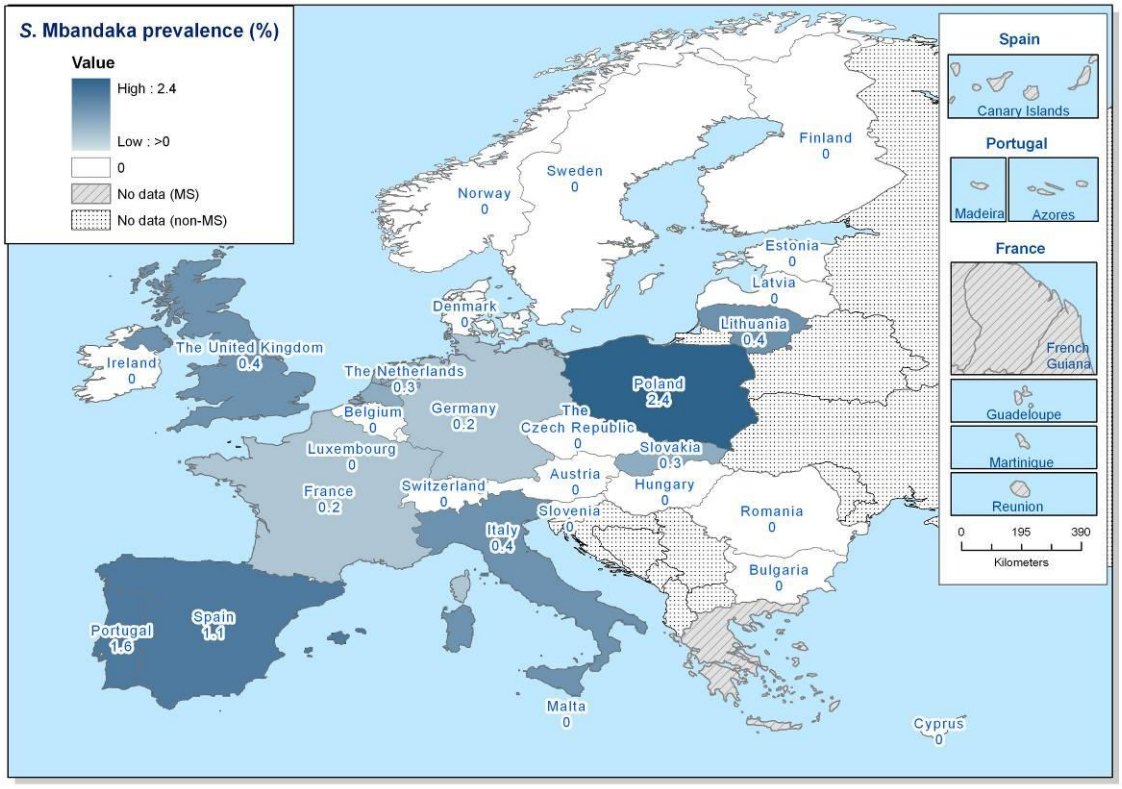

Figure 29: Distribution of the estimated prevalence of *S.* Mbandaka in countries participating in the broiler carcass baseline survey, 2008

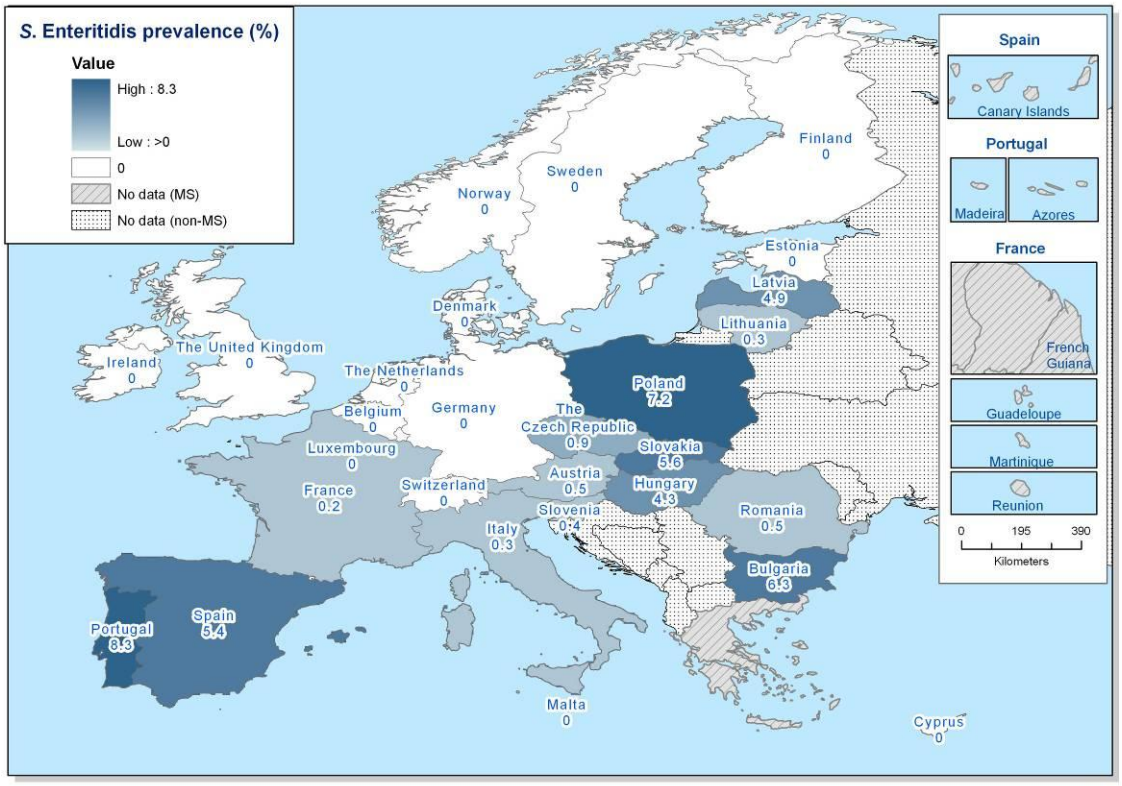

Figure 30: Distribution on the estimated prevalence of *S.* Enteritidis in countries participating in the broiler carcass baseline survey, 2008

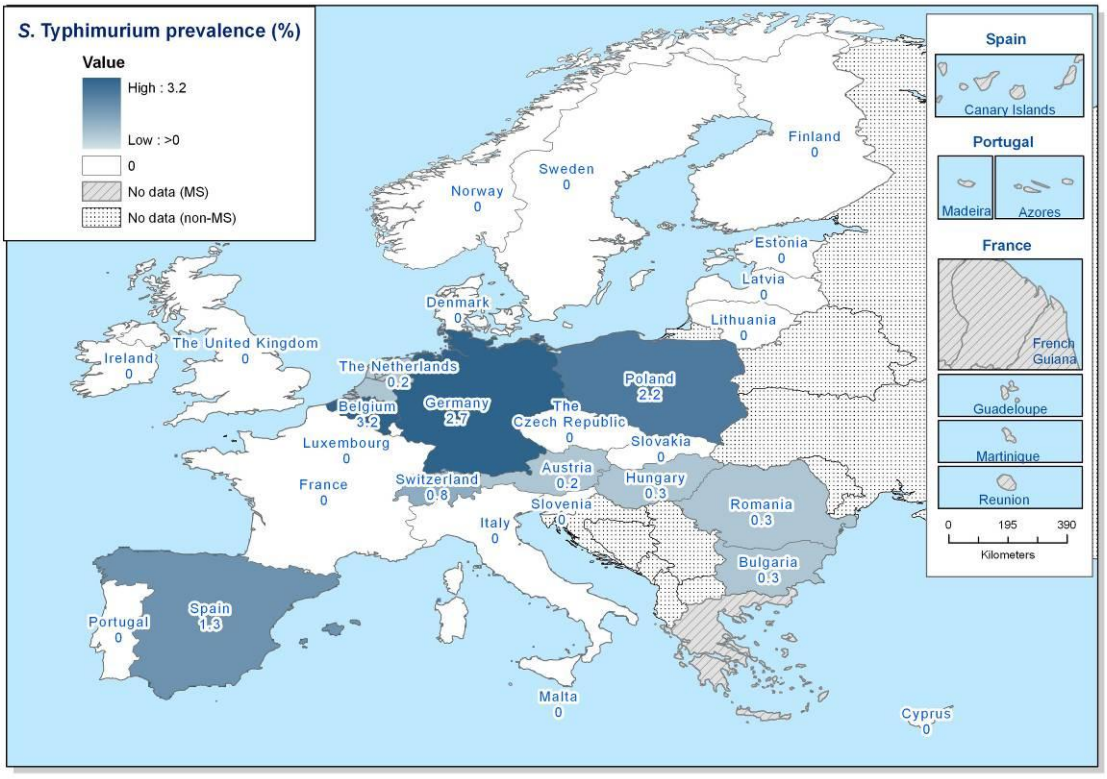

Figure 31: Distribution on the estimated prevalence of *S.* Typhimurium in countries participating in the broiler carcass baseline survey, 2008

ABBREVIATIONS

BS	Baseline Survey
CI	Confidence Interval
CSR	Community Summary Report
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
ICC	Intra-cluster Correlation Coefficient
ISO	International Organization for Standardization
MS(s)	Member State(s)
OR(s)	Odds Ratio(s)
VIF	Variance Inflation Factor