SCIENTIFIC REPORT

Analysis of the baseline survey on the prevalence of *Salmonella* in slaughter pigs, in the EU, 2006-2007

Part B: factors associated with *Salmonella* infection in lymph nodes, *Salmonella* surface contamination of carcasses, and the distribution of *Salmonella* serovars[1]

Report of the Task Force on Zoonoses Data Collection

(Question N° EFSA-Q-2006-042B)

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Summary

A European Union-wide baseline survey was carried out to determine, at the point of slaughter, the prevalence of pigs infected with *Salmonella*, in order to provide the scientific basis for setting a Community reduction target for *Salmonella* in slaughter pigs. The sampling of slaughter pigs took place between October 2006 and September 2007. The pigs were randomly selected from those slaughterhouses that together accounted for 80% of the pigs slaughtered within each Member State. All participating Member States and Norway sampled ileocaecal lymph nodes from the selected slaughtered pigs. Moreover, 13 Member States additionally sampled the corresponding pigs' carcasses by swabbing in order to appreciate the external contamination of the carcasses. A total of 19,159 slaughter pigs with validated results from the European Union and Norway were included in the survey analyses, corresponding to information on 19,025 lymph node samples (from 25 Member States and Norway) and 5,736 carcass swab samples (from 13 Member States).

The analysis of *Salmonella* prevalence was carried out earlier and was published by the European Food Safety Authority on 30 May 2008 in the Part A report. The Community observed prevalence of *Salmonella*-positive slaughter pigs was 10.3%, whereas data from the group of 13 Member States showed that the observed prevalence of carcasses contaminated with *Salmonella* was 8.3% overall. In both cases, prevalence varied among Member States.

In the risk factor analysis, an association between the prevalence of slaughter pigs infected with *Salmonella* in their lymph nodes and the frequency of *Salmonella* surface contamination of the pig carcasses was observed. A *Salmonella* infected pig was twice as likely to yield a *Salmonella* contaminated carcass. However, contaminated carcasses could also derive from uninfected pigs, suggesting potential for cross-contamination in the slaughterhouse environment. The risk of carcasses becoming contaminated with *Salmonella* varied significantly between slaughterhouses even when other associated factors, such as the prevalence of infected slaughter pigs, were accounted for. Moreover, in some slaughterhouses the risks of producing a contaminated carcass both from a *Salmonella* infected pig and from a non-infected pig were significantly higher than in some other slaughterhouses. This indicates that certain slaughterhouses are more capable of controlling and preventing *Salmonella* contamination than others.

The delay between sampling and the start of laboratory testing was found to have an impact on the likelihood of detecting *Salmonella* from the samples. The bacterium was most likely to be detected from lymph nodes and carcass swabs when the sample was tested 3-4 or 1-2 days after sampling, respectively. Also the probability of detecting *Salmonella* from a lymph node sample augmented when the weight of the sample increased.

At the European Union level, the carcasses were less at risk of being contaminated during the first months of the survey, October 2006 to March 2007, compared to the rest of the survey period, from April to September 2007.

The analyses also revealed that there is considerable variation between the significant factors associated with *Salmonella* infection in slaughter pig's lymph nodes, or *Salmonella* carcass contamination, among Member States and also when compared to EU level.

A tendency towards Member State-specific clusters of *Salmonella* serovars was identified for *Salmonella* infection in slaughter pigs, and spatial distribution of serovars was very heterogeneous. *S*. Typhimurium and *S*. Derby were widespread and dominant in the Member States. However, *S*. Enteritidis was relatively prevalent in some eastern EU Member States.

The descriptive analysis of the serovar distribution supported the notion that pig meat contributes to human *Salmonella* infection. However, many serovars isolated from slaughter pigs in this survey are also common in other food producing animal species and food thereof, indicating that the potential for the contribution to human infections is shared between different sources.

It is recommended that Member States would consider the factors found to be associated with *Salmonella* infection in slaughter pigs and carcasses in this survey when they are designing their *Salmonella* control programmes for slaughter pigs. Control measures both at primary production and at slaughterhouse level should be included in the programmes. In particular sampling and testing procedures need standardisation to enhance sensitivity and comparability of monitoring results.

Member States and the EU pig meat industry are encouraged to develop and enhance *Salmonella* controls in primary production and at slaughterhouses in order to prevent and reduce the contamination of pig carcasses with *Salmonella*. Member States are also invited to perform further studies at national level to identify specifically the risk factors for *Salmonella* infection of slaughter pigs and surface contamination of carcasses.

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1. Introduction

This report describes the results of a baseline survey carried out in the European Union (EU) to estimate the prevalence of *Salmonella* in slaughter pigs. This survey was the fourth in a series of baseline surveys of *Salmonella* carried out within the EU. The objective of the surveys has been to obtain comparable data for all Member States (MSs) through harmonised sampling schemes.

The European Commission has asked the European Food Safety Authority (EFSA) to analyse the results at the survey. In EFSA the task was assigned to the Task Force on Zoonoses Data Collection.

According to Regulation (EC) No 2160/2003 (EC, 2003) on the control of *Salmonella* and other zoonotic agents, which aims to reduce the incidence of food-borne diseases in the EU, results of the survey will enable the setting of the Community target for the reduction of the prevalence of *Salmonella* infection in slaughter pigs.

A report from the Task Force on Zoonoses Data Collection on the "Analysis of the baseline survey on the prevalence of *Salmonella* in slaughter pigs in the EU, 2006-2007, part A: *Salmonella* prevalence estimates" (EFSA, 2008a) was issued on 30 May 2008. That report included the analyses of the prevalence of *Salmonella* in slaughter pigs, the most frequent *Salmonella* serovars reported and the impact of the sampling design.

The present Part B report contains analyses of the effects of potential risk factors for *Salmonella* infection in pigs and contamination of pig carcasses. Further analyses of the distribution of the serovars and phage types of *Salmonella* isolates are also included. Objectives, sampling frame, diagnostic testing methods, as well as data collection, evaluation, reporting and timelines of the baseline survey are specified in Commission Decisions 2006/668/EC (EC, 2006) and 2007/219/EC (EC, 2007) concerning a baseline survey on the prevalence of *Salmonella* in slaughter pigs.

2. Objectives

The objectives of the EU-wide baseline survey on *Salmonella* in slaughter pigs are described in detail in the Part A report.

The specific objectives related to this Part B report are:

- to investigate the effect of factors, which may be associated with *Salmonella* infection of slaughter pigs in the ileo-caecal lymph nodes, at the EU level and for each MS individually,
- to investigate the effect of factors, which may be associated with *Salmonella* surface contamination of slaughter pig carcasses, at the level of a group of 13 MSs, that reported the information, and for each of those MSs individually,
- to investigate the association between the results from bacteriological test of lymph node and the results from bacteriological test of carcass swab, with respect to *Salmonella*,
- to investigate the *Salmonella* serovar distribution in slaughter pigs across the EU, and
- to analyse the information submitted by MSs regarding *S.* Enteritidis and *S.* Typhimurium phage types isolated from slaughter pigs.

The analyses of the antimicrobial susceptibility of *Salmonella* isolates from the survey will be specifically addressed in a separate report to be published later by EFSA.

3. Materials and methods

A detailed description of the design of the baseline survey, sampling design, sample size and bacteriological testing can be found in Annex I of Commission Decision 2006/668/EC of 29 September 2006 (EC, 2006) concerning a financial contribution from the Community towards a baseline survey on the prevalence of slaughter pigs to be carried out in the MSs, and in the Part A report.

3.1. Data description

A detailed description of the validation and cleaning of the dataset carried out is provided in the Part A report. The final dataset contained data from 19,159 slaughter pigs (from 25 MSs and Norway), together with information on 19,071[1] lymph node samples (from 25 MSs and Norway) and 5,736 carcass swab samples from 13 MSs.

In each participating country, a representative sample of carcasses (of market-age pigs weighing between 50 and 170 kg) was randomly selected in slaughterhouses representing at least 80% of domestic production. In order to assess the infection status of slaughter pigs, a 25 gr. sample from an aggregate of ileo-caecal lymph nodes were collected from each carcass. A complementary instruction further indicated that some additional lymph nodes of the distal jejunal chain were to be sampled, if necessary, to complete the weight of the sample up to 25gr. At the laboratory, all lymph nodes of the sample were pooled and analysed for the detection of *Salmonella*. In addition, 13 MSs sampled swabs from the surface of the same carcasses in order to determine the *Salmonella* contamination at the end of the slaughterline. An area of 400 cm^2 of the carcass surface was swabbed in a standardised way.

Certain MSs also conducted serological examination of meat juice or blood samples from the slaughter pigs. However, as explained in the Part A report, no meaningful analysis of this data could be conducted because different assays and cut-off values were used by the MSs. As these serological results were not comparable between MSs, no further analysis was carried out for the Part B report.

In the analysis for this Part B report, Norway is included in the EU level analysis dataset.

[1] In total, 46 lymph node samples originating from 5 countries were discarded due to missing crucial covariate information.

3.2. Analysis of factors associated with *Salmonella* positivity

The general assumptions and framework of the statistical analysis carried out are reported in detail in the Part A report. The observed prevalence[1] of infected slaughter pigs and of contaminated carcasses was defined as the proportion of positive slaughter pigs, or, as the proportion of positive carcasses processed over the one-year period of the baseline survey in MSs.

The effect of potential factors on *Salmonella* positivity was analysed at slaughter pig/carcass level. A slaughter pig was considered infected if microbiological culture of the lymph node sample detected *Salmonella*, otherwise it was considered negative. A carcass was considered contaminated, if microbiological culture of the carcass swab detected *Salmonella*, otherwise it was considered negative.

3.2.1. Definition of the outcome variables

Data on slaughter pig *Salmonella* infection in lymph nodes and on *Salmonella* contamination of carcasses were separately analysed and positivity for *Salmonella* spp. (hereafter *Salmonella*) was the considered outcome.

In the Part A report, the prevalence of any *Salmonella* serovar was reported as *Salmonella* spp. and in addition, the prevalence of *S*. Typhimurium, *S*. Derby, and *Salmonella* serovars other than *S*. Typhimurium and *S*. Derby were analysed separately. The analyses for this Part B report also examined each of these outcomes separately but no important differences were observed compared to the results for the *Salmonella* spp. outcome. Therefore, the results of the analyses of factors associated with the detection of *S*. Typhimurium, *S*. Derby or *Salmonella* serovars other than *S*. Typhimurium and *S*. Derby are not presented.

3.2.2. Factors investigated

Information on factors potentially associated with *Salmonella* positivity was collected by the competent authorities or under their supervision at the time of sampling. The mandatory fields in the questionnaire included factors that could be associated with the outcome variables. The following factors, described in detail in Annexes II and III, were considered:

Factors potentially associated with *Salmonella* infection in slaughter pigs:

- Factors related to the sensitivity of the sampling and testing process
    1. Weight of the lymph node sample
    2. Number of lymph nodes in the sample
    3. Time between the date of sampling and the date of testing in the laboratory
- Factors related to lymph node infection
    4. Month of sampling
    5. Hour of sampling in the slaughterhouse
    6. Weight of carcasses

[1] In this report the observed prevalence means the prevalence estimate that accounts for clustering and weighting but not for imperfect test sensitivity or specificity.

Factors potentially associated with *Salmonella* surface contamination of carcasses:

- Factors related to the sensitivity of the sampling and testing process
  1. Time between the date of sampling and the date of testing in the laboratory
- Factors related to the surface contamination of carcasses
  2. Status of the lymph node sample with respect to *Salmonella* infection
  3. Month of sampling
  4. Hour of sampling in the slaughterhouse
  5. Weight of the carcasses

Some additional 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 scarce data reported.

3.2.3. Exploratory analysis of potentially associated factors

The compulsory information that was recorded about each sample describes factors, or variables, that might be associated with the presence of *Salmonella* in lymph node samples or on carcass swab samples. Categorical variables were analysed through frequency tables and bar graphs. Multiple bar graphs, by MS and for the EU global data, were produced by lattice package 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. Box plots were used for graphical visualisation.

The association between each factor and the status of the sample with respect to *Salmonella* infection/contamination was visually explored by:

a) multiple bar graphs of weighted frequency counts of *Salmonella* positive and negative slaughter pigs, by MS and different levels of categorical variables;

b) bar graphs of *Salmonella* prevalence and 95% confidence intervals, by different levels of categorical variables;

c) box plots of quantitative variables for *Salmonella* positive and negative samples.

In the above bivariate analyses, the possible association between each of the individual factors and *Salmonella* infection/contamination was considered.

In addition, the association between the proportion of *Salmonella* positive carcasses and the proportions of positive pigs in lymph node samples in the same slaughterhouse was visually investigated by Bland-Altman and box plot graphs. Only the slaughterhouses (n=146), for which the number of pigs sampled was greater than 10, were included in this exploratory analysis.

3.2.4. Analysis of multicollinearity among potentially associated factors

The data were further analysed for evidence of association among potentially associated factors, since they may be correlated 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, explained in the section on regression analysis). Essentially, each potential risk factor is used as the outcome in a regression analysis (described in

detail in Annex I, section I.3.1.). A VIF value that equals 1 indicates that there is no correlation among risk factors, whereas VIF values higher than 1 indicate a correlation. A VIF value exceeding 10 is interpreted as an indication of strong multicollinearity.

3.2.5. Identification of factors associated with *Salmonella* positivity

Multiple regression analysis was applied to obtain estimates of the association between each factor, adjusted for the effect of other factors (potential for confounding[1]) and *Salmonella* infection of the ileo-caecal lymph nodes of slaughter pigs or surface contamination of carcasses with *Salmonella*. Multiple regression analyses were carried out at the EU level and separately by MS.

3.2.5.1 Statistical model

Given the use of a binary outcome variable (*Salmonella* positive or negative status) taking 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 done in prevalence estimation (Report part A), certain data characteristics needed to be taken into account in the analysis.

Firstly, certain slaughter pigs/carcasses, which were the epidemiological units of the analysis, were slaughtered at the same slaughterhouse. Therefore, they were exposed to the same conditions and to certain same risk factors, including those on which no information was available in the current survey but that might have been associated with *Salmonella* infection/contamination. Pigs slaughtered in the same slaughterhouse are more likely to have been submitted to similar rearing and pre-harvest processes, including comparable managerial and hygiene practices of farming, transportation, and lairage. Similarly, carcasses processed in the same slaughterhouse are bound to be exposed to similar risk factors for surface contamination associated with the slaughter process. It was, therefore, reasonable to believe that slaughter pigs/carcasses processed at the same slaughterhouse could not be considered as independent observations in statistical analysis. Consequently, correlation among outcomes in those pigs/carcasses slaughtered at the same slaughterhouse was taken into account in the regression models. Possible country confounding effects were also taken account of in the analysis.

For the analysis of risk factors for slaughter pig infection a model was fitted where the effect of slaughterhouse was included as random (random intercept logistic regression) and the effect of the country as a country-specific fixed effect. The assumption underlying this type of model is that each slaughterhouse, and consequently each slaughter pig processed in the slaughterhouse, is

[1] In bivariate analysis, a potential risk factor might appear to be associated with *Salmonella* infection solely due to its association with another risk factor for the infection. If, for example, slaughter pigs from MSs with high prevalence were mostly sampled in summer months, summer could result as strongly associated with *Salmonella* when analysing data at EU level. In this case, conclusions on a strong seasonality of the infection could be drawn, although it was just the effect of unbalanced sampling. In fact, in this example, season may not have any real effect on *Salmonella* infection. Confounding is, therefore, the over- or under- estimation of the effect of a potential risk factor due to its association with other risk factors. In the example, the effect of season was overestimated due to the confounding effect of MS. In order to eliminate confounding, and to obtain valid estimates of the effect of season, an adjustment for MS is necessary, which can be achieved by multiple 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.

characterised by a certain baseline level of risk of infection, regardless of the exposure to risk factors considered in the survey. The inclusion of a country-specific effect, which consists in modelling a different parameter for each country in the model, is an attempt to correct confounding between factors and country. A logistic mixed model, with a slaughterhouse random effect on the intercept and country-specific fixed intercept, was therefore used to detect and assess the effects of risk factors for *Salmonella* infection at slaughter pig level.

In a comparable way, to detect and assess the effects of risk factors for contamination of carcasses with *Salmonella* a logistic mixed model was fitted at carcass level with a country-specific fixed intercept, a slaughterhouse random intercept and random slope for predictor whose effect was expected to vary across slaughterhouses. Inserting a random effect of the slaughterhouse on the slope of a predictor allows the effect of that predictor on the risk of contamination to vary between slaughterhouses, in addition to the baseline level of risk varying between slaughterhouses (random intercept). More detailed explanations on analytical methods are given in Annex I.

Secondly, the sampling design of the survey was stratified. Slaughter pigs were sampled from slaughterhouses that, in turn, were sampled from MSs. Slaughterhouse and MS can, therefore, be considered as strata. The proportion of sampled slaughterhouses was not constant across MSs. Similarly, the proportion of sampled pigs was not constant across slaughterhouses. Therefore, the analysis had to be weighted in order to account for the stratified design and the varying proportion of throughput from each slaughterhouse that was sampled, in order to obtain an unbiased estimate of the association between possible risk factors and *Salmonella* infection of lymph nodes or contamination of carcass surface. This approach was also followed when calculating prevalence (Part A report). The weight to account for the sampling fraction of pigs within slaughterhouses (WY2) was calculated as the ratio between the reported number of pigs produced in a slaughterhouse during a year and the number of sampled pigs in the same slaughterhouse. The weight to account for unequal sampling of slaughterhouses within a MS (WY1) was a proxy-weight calculated as the ratio between 80 percent of the annual domestic throughput of slaughter pigs in the MS and the sum of the annual throughputs of the sampled slaughterhouses in the same MS (Annex I).

3.2.5.2 Model building for *Salmonella* lymph node infection at the EU- and country level

The investigation of the association between factors and the *Salmonella* infection in slaughter pigs (lymph node samples) at EU level was done using a starting model that contained a global intercept, a country-specific fixed effect, the factors of interest, and a random intercept for slaughterhouse. This model was reduced by removing stepwise the most non-significant risk factors until only covariates with *P*-values smaller than or equal to 0.05 remained in the final model. Since no positive results were reported by Finland, it could not be considered in the EU level analysis, as no country-specific effect could be estimated. Bulgaria was also excluded from the global model building because its weight WY1 was not determined.

A similar model building exercise was carried out at country level: for each of the participating countries a separate model was run. As in the EU model building, covariates were selected through a backward selection procedure using random effect logistic regression. A slaughterhouse-specific random intercept was incorporated into the model, which was fitted using the GLIMMIX procedure in the SAS® System. The model for each country was then further reduced so that only covariates with *P*-values smaller than or equal to 0.25 remained. Further, for certain countries (Austria, Cyprus, Ireland, Sweden, The Netherlands), the slaughterhouse

random-effect was not taken into account in the logistic regression model, because of specific model fitting obstacles.

3.2.5.3 Model building for *Salmonella* carcass surface contamination at the MS group and MS level

The investigation of the association between factors and carcass *Salmonella* contamination at the level of the group of 13 MSs was also carried out using a backward selection procedure. The starting model contained a global intercept, a MS-specific fixed effect, all potentially associated factors of interest and a random intercept for slaughterhouse. A slaughterhouse random slope was also added for the "Lymph node infection" variable. The model was fitted using the GLIMMIX procedure in the SAS® System. As Slovenia and Sweden had no *Salmonella* positive samples, these MS data were not included in the analysis because no information was available to estimate the country-specific effect. A similar model building exercise was performed on MS level: for each of the participating MSs a separate model was fitted.

3.3. Analysis of the association between slaughter pigs' lymph node *Salmonella* infection and their carcass *Salmonella* contamination

The quantification of the association between the bacteriological culture of lymph node samples and culture of carcass swabs with respect to *Salmonella*, was done by investigating the odds ratio (OR) covered in the final EU level and MS-specific risk factor analyses models for carcass *Salmonella* contamination as mentioned above, with the lymph node sample with respect to *Salmonella* infection as an explanatory variable for the carcass swab outcome.

3.4. Analysis of the serovars and phage types distribution

3.4.1. Spatial distribution of reported *Salmonella* serovars in lymph nodes

The geographical analysis of the *Salmonella* serovar distribution was limited to country level, as the location (coordinates) of the individual pig herds and/or slaughterhouse was not available. The scan statistics (SaTScan™) developed by Kulldorff was applied to detect spatial clusters of MSs where each of the selected serovars was detected. The detection of clusters would allow generating hypotheses on transmission or on common sources of *Salmonella* serovars in slaughter pigs of neighbouring MSs. Moreover, SaTScan also allows for the detection of individual MSs with a significant above EU average risk of *Salmonella*-specific serovar infection in slaughter pigs.

SaTScan uses a circular window of different sizes to scan the study area. For each circle the method computes the likelihood that the risk of infection is higher inside the circle compared to outside the circle. The circle with the highest likelihood value is the one that has the highest probability of containing a cluster. SaTScan accounts for multiple testing through the calculation of the highest likelihood of occurrence for all possible cluster locations and sizes. The Poisson model was chosen, which requires information about the number of estimated positive cases in

each MS and the population data. The estimated number of positive cases for each serovar was calculated from the estimated prevalence. All estimated positive cases were geocoded to the centroid of its respective country. The maximum window size was defined here as 50% of cases and 999 replications were performed. It was set to look for spatial clusters of *Salmonella* spp., of *S.* Derby, of *S.* Typhimurium, *S.* Enteritidis, *S.* Infantis and *S.* Rissen. Only the most likely cluster and non-overlapping significant secondary clusters were displayed in this analysis. For the analysis, the SaTScan output was imported into Arc GIS 9.1 to create cluster maps to visually examine and compare identified clusters.

3.4.2. Comparison between *Salmonella* serovar and phage type distribution in slaughter pigs, other animal species, feed and human salmonellosis cases

The serovar distribution found in ileo-caecal lymph nodes and on carcasses of slaughter pigs was compared with the serovar distribution among MSs in animal feed and in human salmonellosis cases as reported in the Community Summary Report on Zoonoses in 2006 (EFSA, 2006a). It was also compared with serovar distribution among MSs in laying hen holdings, broiler and turkey flocks as reported in previous baseline surveys (EFSA, 2007a; EFSA, 2007b; EFSA, 2008b). Phage type distribution was described for *S.* Enteritidis and *S.* Typhimurium for lymph node and carcass samples. The descriptive analysis of the serovar and phage type data was performed in Microsoft Excel.

4. Results

4.1. Analysis of factors associated with *Salmonella* infection in lymph nodes of slaughter pigs

In the following, the results are presented of the univariate description of potentially associated factors and of the bivariate association between potentially associated factors, and *Salmonella* infection in slaughter pigs, as determined by lymph node analyses. The graphs presenting the bivariate associations must be considered as exploratory data analysis because these associations have not been adjusted for the effect of other factors (potential for confounding) and for the MSs' effects. Following the bivariate analysis, results from the multiple regression analysis are presented, which are adjusted for the recorded confounding variables, notably country effect.

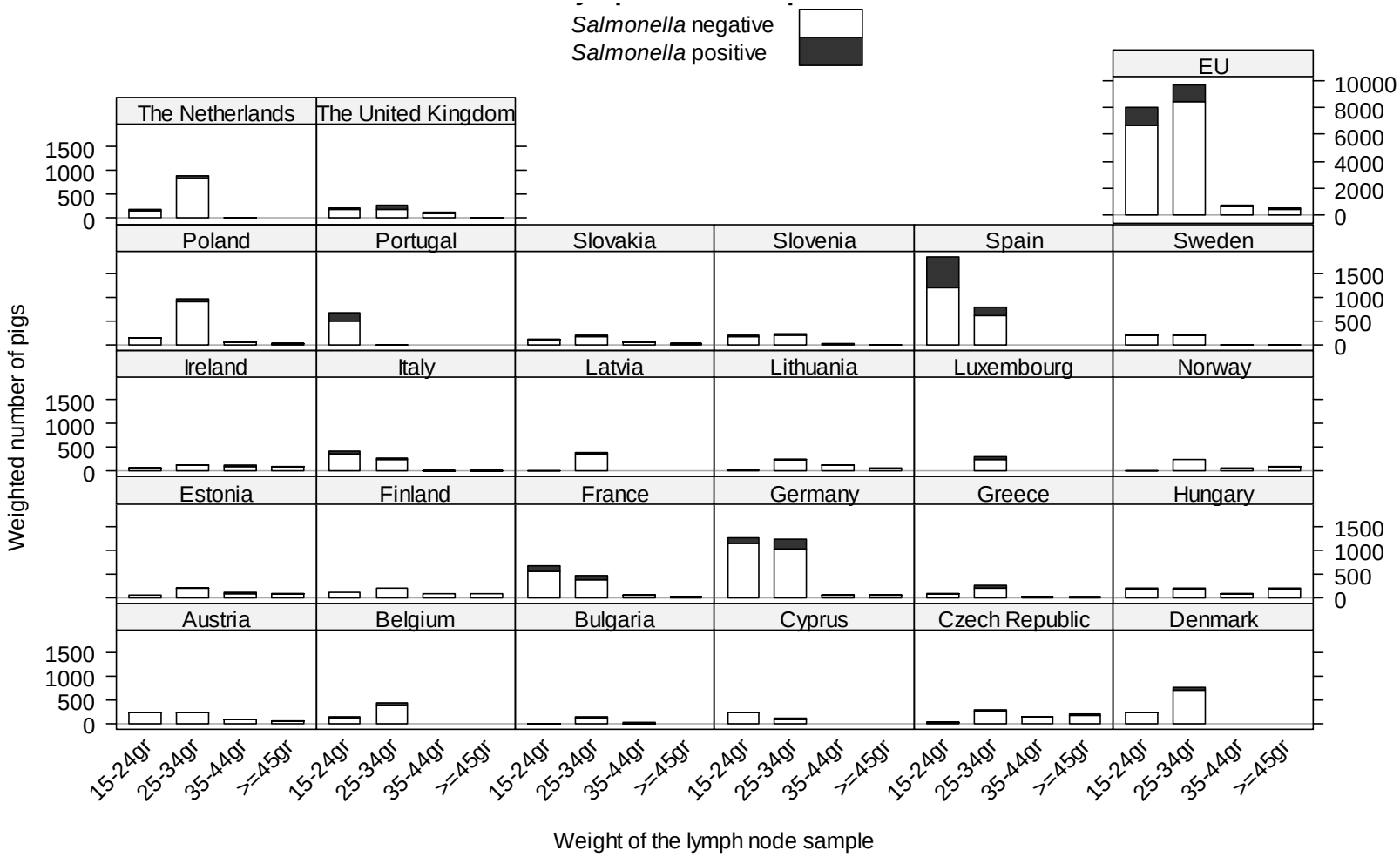
4.1.1. Descriptive analysis of factors potentially associated with *Salmonella* infection

4.1.1.1 Factors related to the sensitivity of the sampling process

- *Weight of the lymph node samples*

A graphical display of the total weight of sampled lymph nodes by MS is presented in Figure 1. This graph, as in similar ones presented hereafter, displays weighted frequencies (Annex II - Tables II.1 and II.2). This means that the weighting of each pig was taken into account to show a balanced prevalence within each month. Most lymph node samples (89%) belonged to the first two weight categories: between 15 and 24gr and between 25 and 34gr, whereas only 11% of the lymph node samples weighed more than 34gr. *Salmonella* positive lymph node samples belonged mostly to the 15-24gr category, probably due to Spain's strong contribution. The impact of the weight of the lymph node samples on *Salmonella* detection in slaughter pigs has to be assessed taking into account MS effect – refer to section 4.1.3.

Figure 1. Bar plot of the weight of the lymph node samples tested, by country and for the EU, and by *Salmonella* status

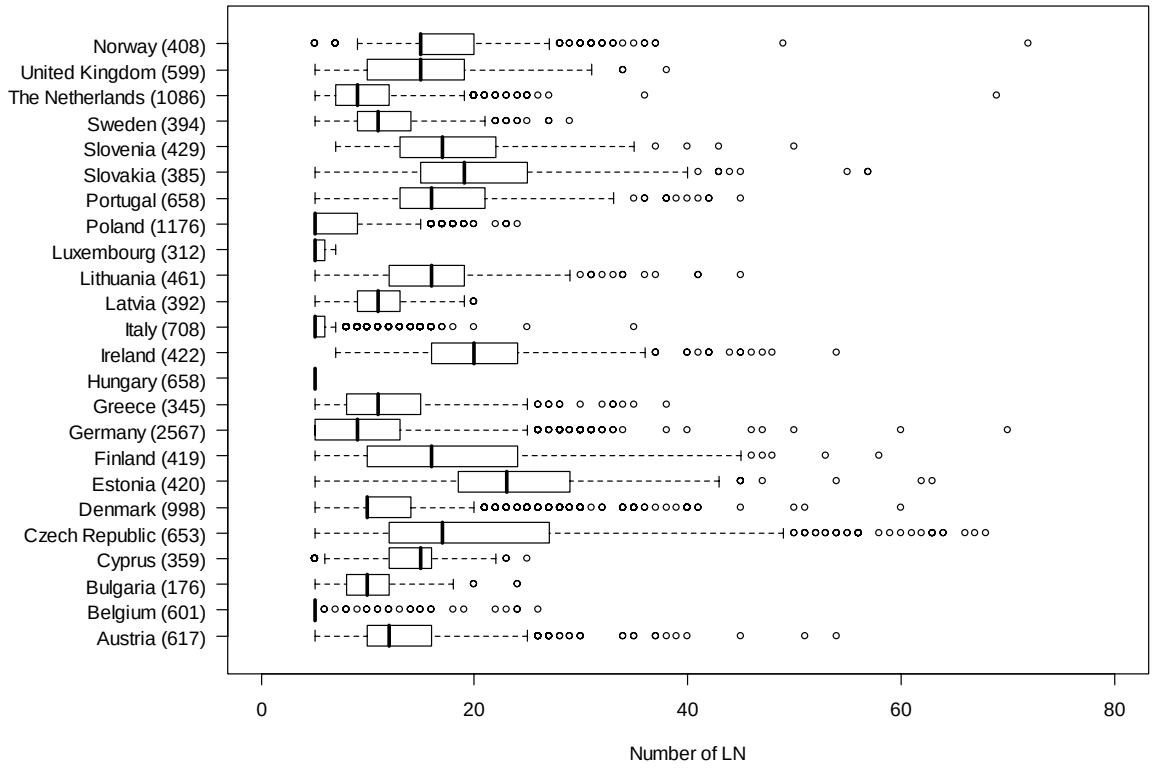


- *Number of lymph nodes in the sample*

Figure 2 presents a graphical display of the number of lymph nodes per sample within MSs and Norway, by means of box plots[1]. At EU level, the mean was 16.6 and the median (Q1; Q3) was 10 (6; 16). Medians were highest in Estonia (23), Ireland (20) and Slovakia (19), whereas the lowest median (5) was encountered in Belgium, Hungary, Italy, Luxembourg and Poland. Descriptive statistics of the number of lymph nodes in samples are presented in Annex II – Tables II.3 and II.4.

Figure 2. Box plot of the number of lymph nodes (LN) per sample, per country

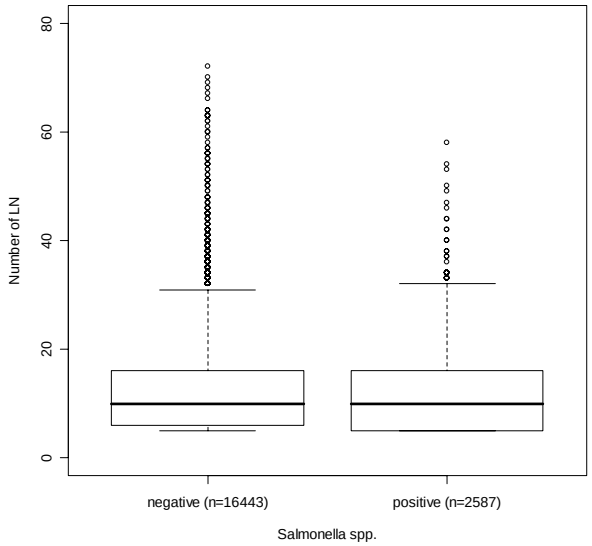
The number of sampled slaughter pigs per MS is indicated between brackets.



The median number of collected lymph nodes per sample was not different for *Salmonella* positive than for *Salmonella* negative ileo-caecal lymph node samples (Figure 3).

[1] In the horizontal box plots, the left of the box represents the first quartile (Q1) of the distribution and the right the third quartile (Q3), whereas the bar inside the box represents the median. Small circular symbols indicate extreme values, differing from the box > 1.5 times the difference between the third and the first quartile (interquartile range).

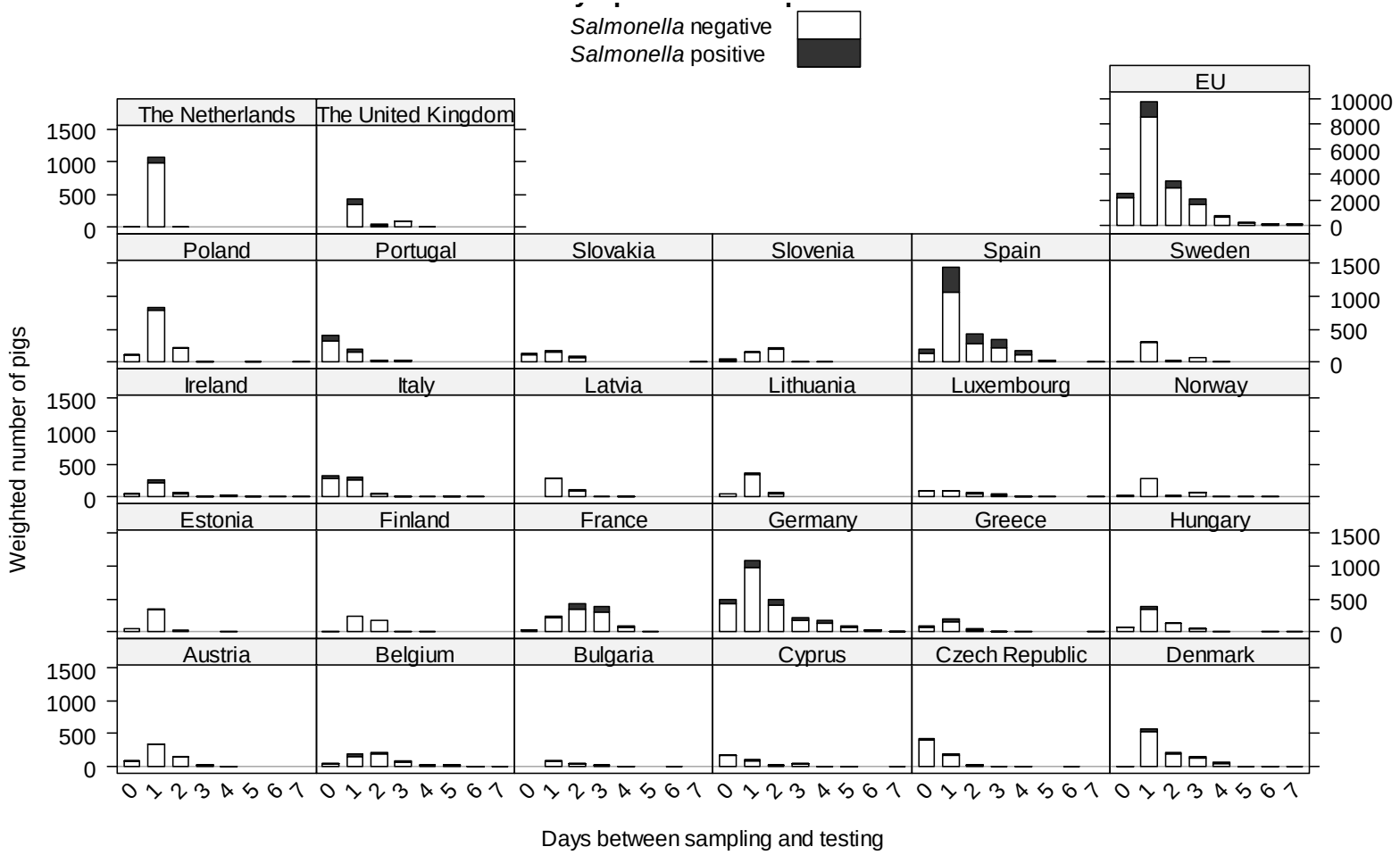
Figure 3. Box plot of the number of lymph nodes per sample by *Salmonella* status of sample



- *Time between the dates of sampling and testing in the laboratory*

The time between the date of sampling and the date of testing in the laboratory varied among MSs (Figure 4 and Annex II - Table II.5). Most lymph node samples (53%) were analysed for *Salmonella* 1 day after sampling. Eighty-six percent of the samples were tested between 0 and 2 days after sampling.

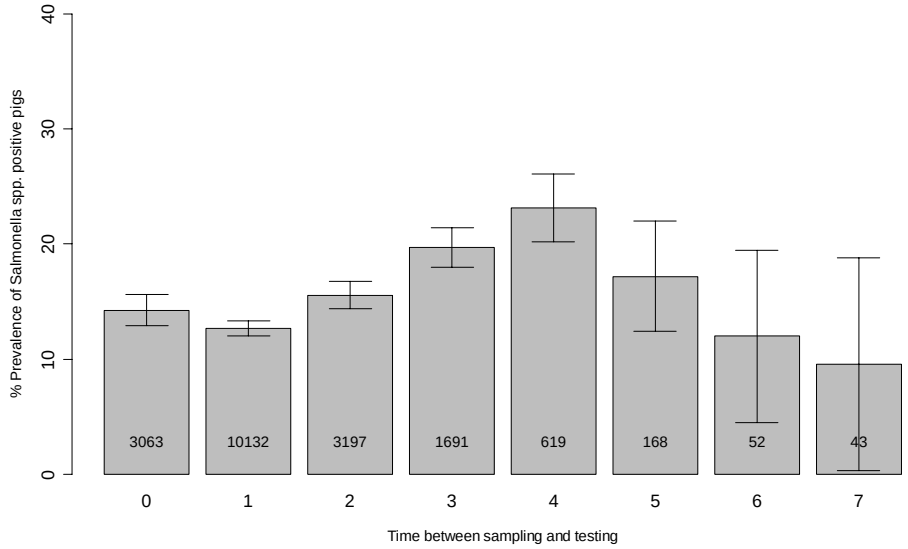
Figure 4. Frequency distribution of the weighted number of days between sampling and testing of lymph node samples, by country and for the EU, and by *Salmonella* status



In general, there was an increase in *Salmonella* prevalence associated with an increased number of days between sampling and testing up to a delay of 4 days in testing, followed by a decrease for a delay of over 4 days (Figure 5, and Annex II – Table II.6) up to the accepted maximum of 7 days.

Figure 5. Weighted *Salmonella* prevalence by number of days between sampling and testing, with 95% confidence intervals, in the EU

The number of sampled pigs is indicated inside each bar.



As no linear trend was observed, the "time between the date of sampling and testing" variable was categorised into 3 levels for further analyses: 0-2 days, 3-4 days and 5-7 days. Categorisation results are shown in Annex II – Tables II.7 and II.8.

4.1.1.2 Factors related to the lymph node infection

- *Month of sampling*

A graphical display of the number of lymph node samples collected at MS-specific and at EU level every month during the survey is presented in Figure 6 (see also Annex II – Tables II.9). The collection of lymph node samples in slaughter pigs was homogeneous during the survey for most participating countries. However, Bulgaria, Latvia, Lithuania and Portugal were delayed in the start of sampling. The number of lymph node samples peaked at the EU level in September 2007 largely due to the contribution of Hungary, Poland and Spain, where most samples were taken in that month. Denmark and France also contributed to the peak. *Salmonella* prevalence seems to be lower during the first two months of the survey (October - November 2006) compared with the following months of the survey. A slight increasing trend in prevalence is also suggested by the graphical visualisation of the data, from January to the summer months of 2007 (Figure 7, Annex II – Table II.10).

Figure 6. Bar plot of the weighted number of lymph node samples collected by month and country, and for the EU, and by *Salmonella* status

Months are ordered from October 2006 to September 2007.

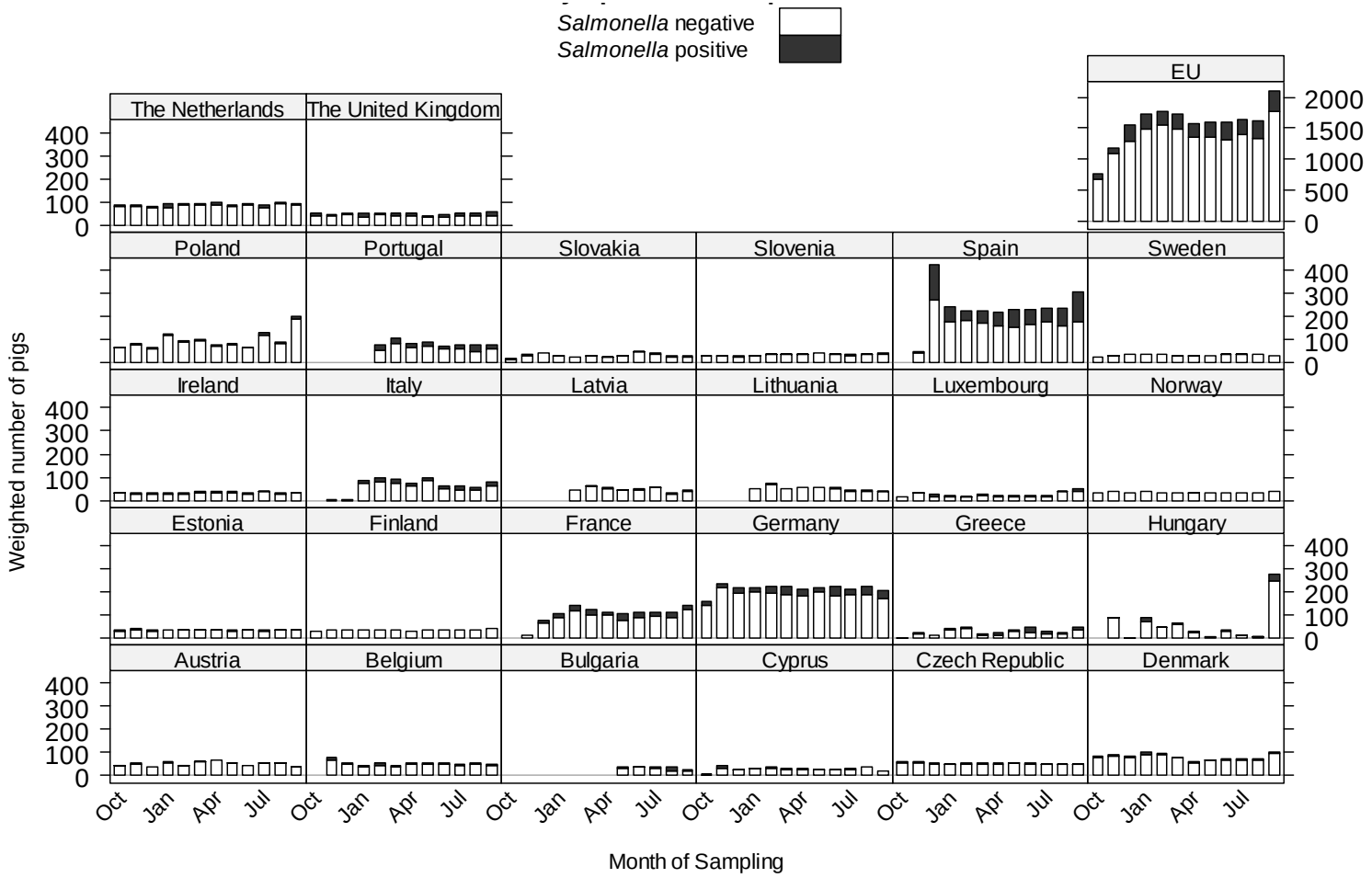
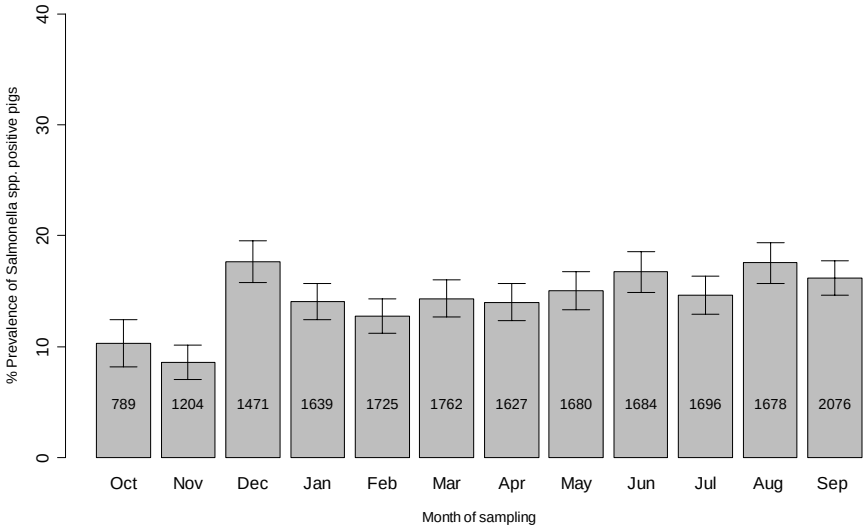


Figure 7. Weighted *Salmonella* prevalence by the month of sampling, with 95% confidence intervals, in the EU

The number of sampled pigs is indicated inside each bar.

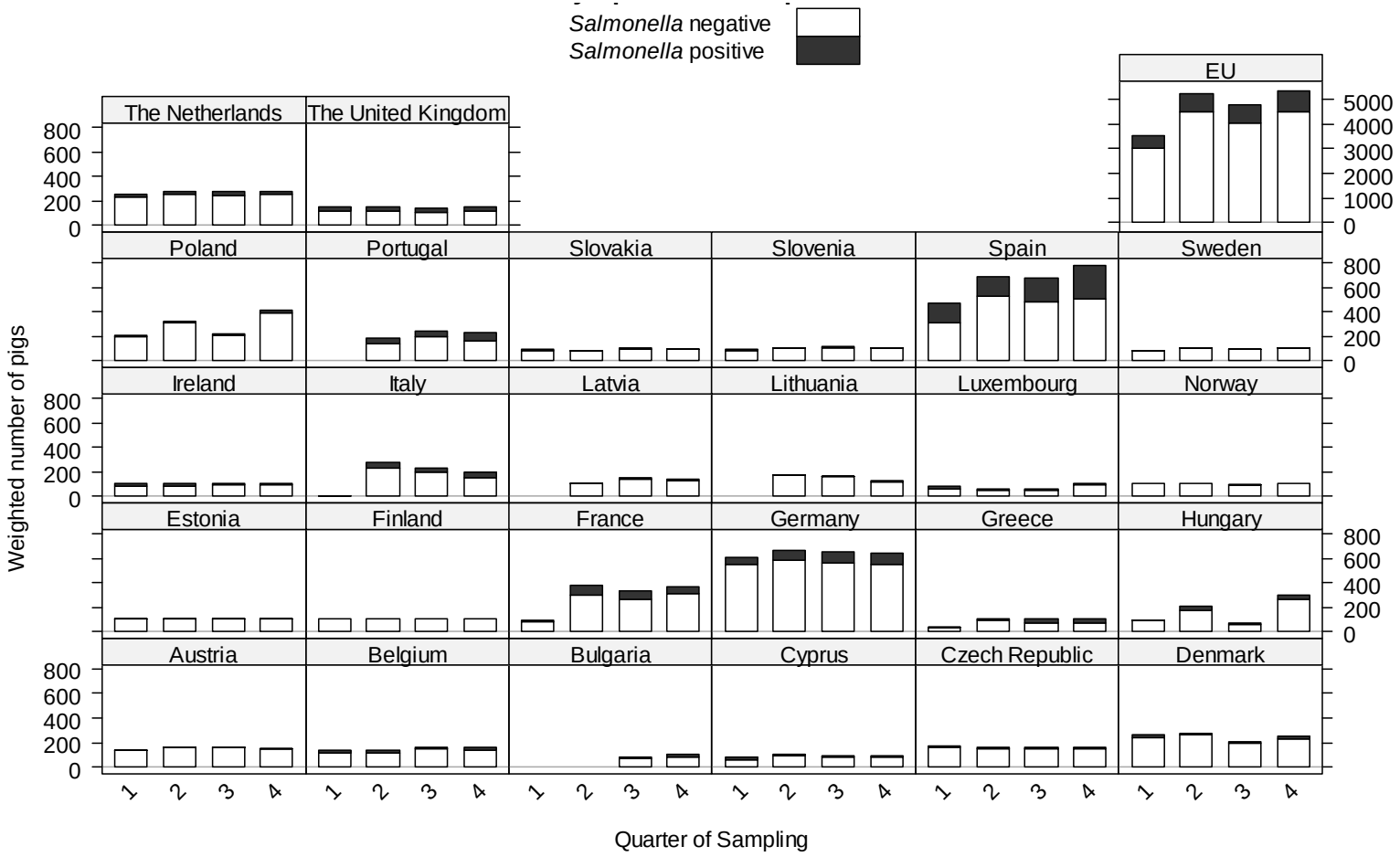


- *Sampling quarter*

Treating the month of sampling as a categorical variable implies a nominal variable with 12 categories. Including all months as categories of a class variable may yield to over-parameterisation of the multiple regression model, especially when countries are considered separately. To remedy this problem and because a seasonal trend could be expected to occur, a categorical variable "Sampling quarter" was created with the following four categories: October-December 2006, January-March 2007, April-June 2007, and July-September 2007. In order to test for any seasonal effect on the risk of *Salmonella* infection in slaughter pigs, the four categories were coded: 1 when the slaughter pig was sampled in the period October-December 2006, 2 when sampled in the period January-March 2007, 3 in the period April-June, and 4 in the period July-September 2007. A graphical display of the numbers of lymph node samples collected at MS-specific and at EU level in each quarter during the survey is presented in Figure 8 (see also Annex II – Table II.11).

Figure 8. Bar plot of the weighted number of tested lymph node samples, by sampling quarter and country, and for the EU, and by *Salmonella* status

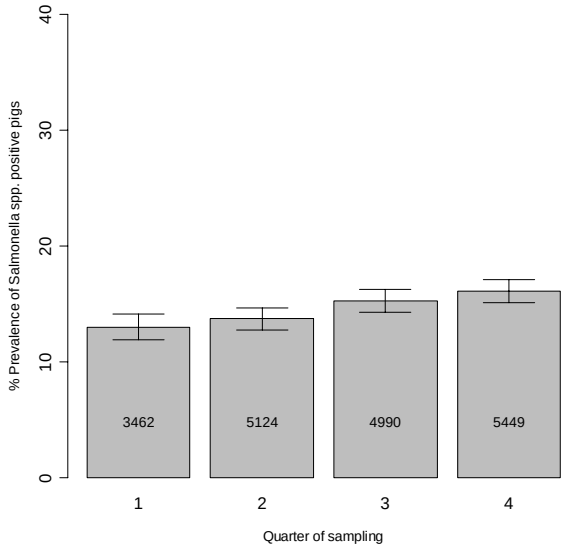
Quarters are ordered from October-December 2006 (1) to July-September 2007 (4).



Generally, *Salmonella* prevalence in lymph nodes appears to increase towards the end of the survey (Figure 9, see also Annex II – Table II.12). However, care must be taken in interpreting this observation, as there were substantial differences among the MSs in the distribution of samples across the quarters of the sampling period. Therefore, confounding is possible.

Figure 9. Weighted *Salmonella* lymph node prevalence by sampling quarter, with 95% confidence intervals, in the EU

Quarters are ordered from October – December 2006 (1) to July —September 2007 (4).
Number of sampled pigs represented inside each bar.



- *Hour of sampling*

A graphical display of the number of samples collected at country-specific and at EU level during each hour of the working day in slaughterhouses is presented in Figure 10.

Figure 10. Bar plot of the weighted number of lymph node samples collected, by hour of sampling and country, and for the EU, by *Salmonella* status

Hours are ordered from 00 to 23.

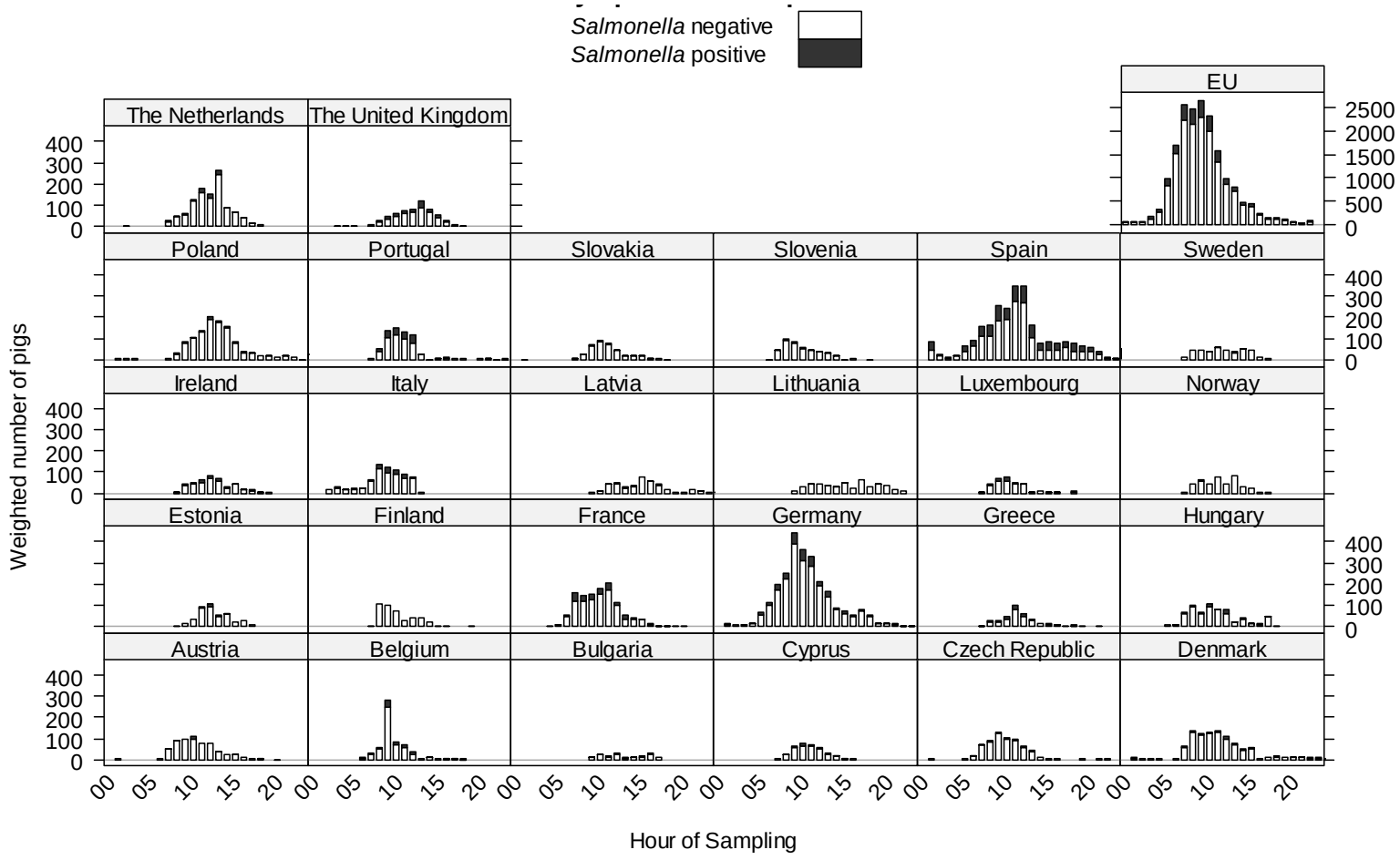
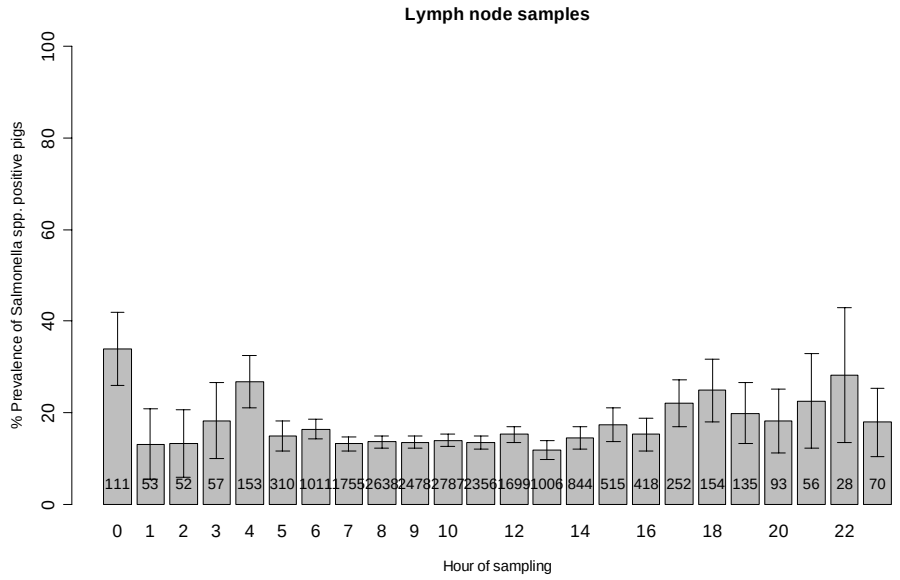


Figure 11 suggests a lower prevalence in lymph node samples during the day time (see also Annex II – Table II.13). However, most samples were taken between 05:00 and 18:00.

Figure 11. Weighted *Salmonella* prevalence by hour of sampling, with 95% confidence intervals, in the EU

Number of sampled pigs indicated inside each bar.



Treating the variable "Hour of sampling" as a categorical variable in the modelling exercise implies a nominal variable with 24 categories, and will result in an additional 23 parameters. As presented in detail in Annex IV (section IV.1), a sine type of evolution of the prevalence of infection can be modelled, with peaks during the night time. The new variable "Time of sampling (sine)" was therefore used in the building of the model on slaughter pig infection. A significant effect of this variable would imply that there is a sine trend such that day and night results differ significantly.

- *Weight of carcasses*

At EU level, the median carcass weight (Q1; Q3) was 85 kg (74; 95). The distribution of carcass weights for slaughter pigs sampled for lymph nodes, at country level is shown in Figure 12 (Annex II – Table II.14). The heaviest carcasses were sampled in Italy (median=132 kg) and Hungary (median=110 kg), whereas the medians were lowest in Cyprus (70 kg), Greece (70 kg), Estonia (72 kg) and Latvia (72 kg). The median weight of carcasses in the group of infected slaughter pigs is lower than the median weight of carcasses of negative slaughter pigs (Figure 13, see also Annex II. – Table II.15). However, this observation must be adjusted for potential confounding before any conclusions are made.

Figure 12. Box plot of carcass weights for slaughter pigs sampled for lymph nodes, per country

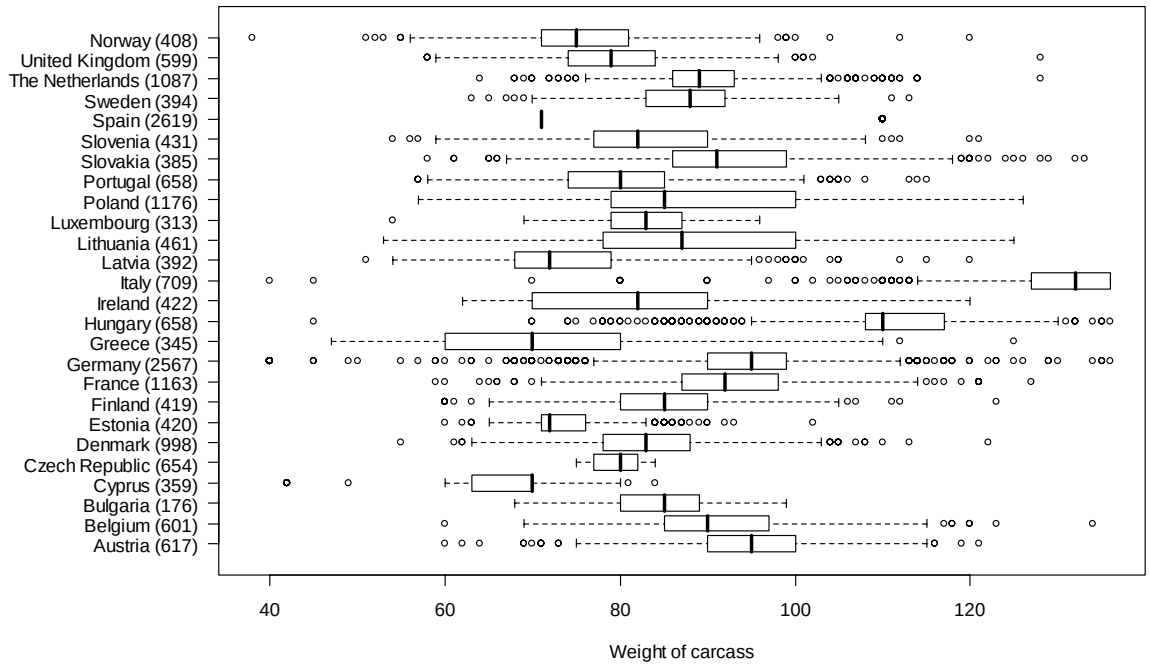
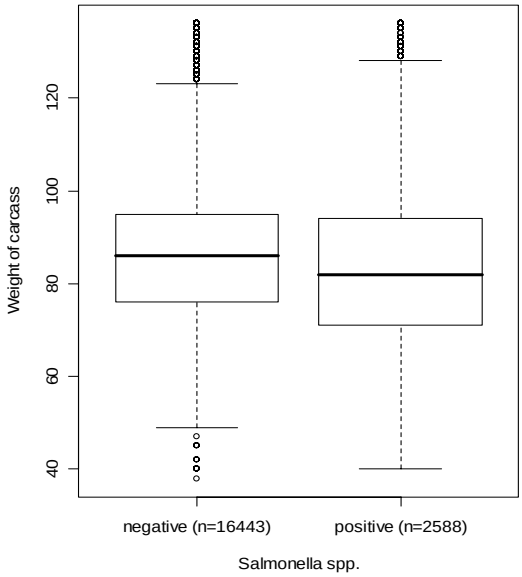


Figure 13. Box plot of carcass weight for slaughter pigs sampled for lymph nodes, by *Salmonella* status



4.1.2. Analysis of multicollinearity among potential factors

The VIF values calculated for the multicollinearity analysis among the factors associated with *Salmonella* prevalence in lymph node samples in the EU are presented in Table IV.1 of Annex IV. This analysis showed that multicollinearity was not important for the global model.

The analysis was repeated focussing on each separate participating country and the VIF values are displayed in Table IV.2 of Annex IV. This analysis showed that multicollinearity was neither an issue for the MS models.

4.1.3. Multiple regression analysis at EU level

In this section, Norway is also included in the EU level analyses and results referring to it. Overall, factors associated with *Salmonella* infection in lymph node samples of slaughter pigs are presented in Table 1. The two factors retained in the final regression model were related to the sensitivity of the sampling process. The model included country-specific effects (Annex IV – Table IV.5). Therefore, each odds ratio[1] (OR) was adjusted for MSs. A random intercept for the slaughterhouses (Annex IV – Table IV.6) was also inserted in the model.

Table 1. The final random effect logistic model for factors associated with *Salmonella* infection in lymph nodes of slaughter pigs, in the EU, 2006-2007.

Variables	Random effect logistic model [a, b]	
	OR	95%CI
Weight of the lymph node samples [c]		
15-24gr	1	-
25-34gr	1.3	1.1, 1.6
35-44gr	1.2	0.8, 1.7
≥ 45gr	1.9	1.2, 3.0
Time (in days) between the date of sampling and testing in the laboratory [c]		
0 to 2 days	1	-
3 to 4 days	1.2	1.04, 1.4
5 to 7 days	0.99	0.65, 1.5

[a] Estimates and standard errors were assessed using a mixed model with a slaughterhouse random effect on the intercept and country-specific fixed intercept.

[b] As the country-specific effect of Finland could not be estimated (no lymph node samples tested positive for *Salmonella*), that Member State was not considered in the EU level analysis.

[c] Significant at *P*-value < 0.05.

[1] An OR of 1.0 implies that there is no association between a risk factor and *Salmonella* infection; an OR above 1.0 implies an increased risk of *Salmonella* infection among pigs exposed to that factor while an OR below 1.0 implies a reduced risk of *Salmonella* infection among exposed pigs. In any study, it is possible that an OR different to 1.0 may arise by chance and the level of significance (*P*-value) estimates this probability. Consequently, if the 95% confidence interval 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 risk factor and *Salmonella* is statistically significant ($P < 0.05$).

According to the analyses, the probability of *Salmonella* detection in lymph nodes increases as the weight of the lymph node samples increases. For example, the odds of detecting *Salmonella* infection in a lymph node sample weighing more than 45gr is 1.9 times higher than the odds for a 15-24gr sample. The effect of the time delay between sampling and the start of laboratory testing is also identified as a factor associated with *Salmonella* detection in lymph nodes. A 3-4 day delay in testing increases the likelihood of detection of *Salmonella* in lymph node samples by 20% compared to a delay of less than 2 days. Conversely, the impact of a delay of over 4 days on the risk of *Salmonella* detection was not significantly different from a delay of less than 2 days. However, only few samples were examined more than 4 days after sampling.

4.1.4. Multiple regression analysis at the country level

Results of regression analysis by country are presented in Table 2, where rows correspond to country and columns to potential associated factors. Cells in the table contain the OR measuring the risk factor effect in the corresponding column and its 95% CI, in the country in the corresponding row. In addition, the shading of the cells is used to show whether the OR obtained a level of conventional statistical significance. Each level of significance (*P*-value of less than 1%, 5%, 10%, and 25%, respectively) is indicated by a different shade of grey, darker meaning there was a greater probability that the observed association did not arise by chance alone. Empty cells mean that there was insufficient evidence of an association between the risk factor and *Salmonella* infection in that particular country and the risk factor was thus not included in the final model. For some risk factors with more than two categories, data were not available for all categories in some countries. For instance, for Belgium only categories "15-24gr" and "25-34gr" of the variable "Weight of the lymph node samples" were available. Therefore, only these two levels are available for comparison to obtain an OR estimate for this factor for the country.

The matrix presentation of results facilitates the identification of factors that may increase or reduce the risk of *Salmonella* infection across countries, as the effects of these factors might vary among countries. Indeed, a great variability between significant risk factors obtained for each country was observed. Some factors even had contrasting effects depending on the country. In addition, when these effects are studied at EU level, these results may average out so that no significant effect is observed in the general model.

The final models fitted for Austria, Ireland, Latvia, Portugal, Slovenia, Sweden and the United Kingdom did not identify factors among those tested that were significantly associated (at a level of 5% or less) with *Salmonella* infection in lymph nodes. As the model fitted for Norway is based on one positive pig only, the results of this analysis should thus be considered with caution.

Significant associations (*P-value* < 0.05) observed for each of the factors across the MSs are:

- Carcass weights (10 kg increments) – This factor was significantly associated with an increased risk of *Salmonella* infection in three countries (Lithuania, Poland, and Norway); where for every 10 kg increase in carcass weight, the risk increased by 20-60%. However, for two countries (Belgium and Hungary), the increase of carcass weight appeared to be associated with a lower risk of infection.

Table 2. Random effect logistic models for factors associated with *Salmonella* infection in slaughter pigs in participating countries

Odds ratio estimates and 95%CI are presented for significant (at different levels of significance) risk factors obtained for each country separately. The shade of gray of the cell illustrates the level of significance (*P*-value) of the association, according to the following scale:

$P < 0.01$: $0.01 < P < 0.05$: $0.05 < P < 0.10$: $0.10 < P < 0.25$:

	Number of carcasses	Weight of the lymph node samples			Number of lymph nodes in the sample	Time between sampling and testing		Sampling quarter			Sampling time (sine)	Weight of carcasses (10-kg increments)
		25-34gr vs. 15-24gr	35-44gr vs. 15-24gr	≥ 45gr vs. 15-24gr		3 to 4 days vs. 0 to 2 days	5 to 7 days vs. 0 to 2 days	Oct.-Dec.06 vs. Jul.-Sep.07	Jan.-Mar.06 vs. Jul.-Sep.07	Apr.-Jun.06 vs. Jul.-Sep.07		
Austria [a]	617	**6.9** 0.8, 62	**11.5** 1.2, 112	**10.2** 0.8, 125		**3.3** 0.65, 16	N/A					
Belgium	601	**0.6** 0.3, 0.9	N/A	N/A	**0.92** 0.84, 1.01							**0.87** 0.76, 0.99
Bulgaria	176	**0.1** [b] 0.005, 1.7	**0.4** [b] 0.03, 6.7	N/A							**0.06** 0.04, 0.11	
Cyprus [a]	359	**2.3** 1.2, 4.5	N/A	N/A				**4.3** 1.5, 13	**1.8** 0.6, 5.5	**2.6** 0.9, 7.9		**0.62** 0.35, 1.1
Czech Republic	653	**2.2** 0.12, 39.4	**1.05** 0.05, 24.3	**0.9** 0.05, 14.6	**1.03** 1.01, 1.05							**1.8** 0.67, 4.7
Denmark	998	**1.8** 1.07, 2.9	N/A	N/A		**1.5** 1.01, 2.1	**0.7** 0.09, 5.5					
Estonia	420										**0.05** 0.01, 0.5	

Table 2. ***Continued***

	Number of carcasses	Weight of the lymph node samples			Number of lymph nodes in the sample	Time between sampling and testing		Sampling quarter			Sampling time (sine)	Weight of carcasses (10-kg increments)
		25-34gr vs. 15-24gr	35-44gr vs. 15-24gr	≥ 45gr vs. 15-24gr		3 to 4 days vs. 0 to 2 days	5 to 7 days vs. 0 to 2 days	Oct.-Dec.06 vs. Jul.-Sep.07	Jan.-Mar.06 vs. Jul.-Sep.07	Apr.-Jun.06 vs. Jul.-Sep.07		
France	1,163	**1.3** 0.94, 1.7	**0.82** 0.21, 2.9	**4.2** 1.3, 13	N/A							
Germany	2,567	**1.8** 1.2, 2.7	**1.3** 0.45, 3.7	**0.9** 0.32, 2.3								**0.90** 0.79, 1.04
Greece	345							**0.078** 0.010, 0.64	**0.32** 0.14, 0.76	**1.1** 0.40, 2.9		**1.2** 0.93, 1.6
Hungary	658	**1.7** 0.74, 4.0	**1.3** 0.49, 3.5	**2.1** 0.86, 5.1								**0.65** 0.46, 0.91
Ireland [a]	422				**0.98** 0.94, 1.01			**2.6** 1.1, 6.3	**3.0** 1.3, 7.1	**1.9** 0.79, 4.6		
Italy	708	**1.1** [b] 0.67, 1.7	**1.6** [b] 0.75, 3.5	**14** [b] 11, 17								
Latvia	392										**2.2** 0.98, 4.9	
Lithuania	461										**0.02** <0.001, 8.8	**1.6** 1.2, 2.1
Luxembourg	312							**2.1** 1.1, 3.9	**5.9** 0.47, 74	**4.5** 0.36, 57		**0.61** 0.34, 1.1

Table 2. ***Continued***

	Number of carcasses	Weight of the lymph node samples			Number of lymph nodes in the sample	Time between sampling and testing		Sampling quarter			Sampling time (sine)	Weight of carcasses (10-kg increments)
		25-34gr vs. 15-24gr	35-44gr vs. 15-24gr	≥ 45gr vs. 15-24gr		3 to 4 days vs. 0 to 2 days	5 to 7 days vs. 0 to 2 days	Oct.-Dec.06 vs. Jul.-Sep.07	Jan.-Mar.06 vs. Jul.-Sep.07	Apr.-Jun.06 vs. Jul.-Sep.07		
Poland	1,176	**1.1** [b] 0.52, 2.5	**0.56** [b] 0.045, 6.9	**8.7** [b] 1.8, 41				**0.64** 0.31, 1.3	**0.43** 0.20, 0.94	**0.66** 0.32, 1.4		**1.2** 1.03, 1.5
Portugal	658							NA	**0.8** 0.5, 1.3	**0.6** 0.3, 1.1		
Slovakia	385	**0.48** 0.15, 1.5	**0.59** 0.19, 1.8	**1.4** 0.23, 8.3				**0.98** 0.38, 2.5	**0.37** 0.093, 1.4	**0.23** 0.098, 0.5		
Slovenia	429							**1.7** 0.91, 3.0	**0.71** 0.19, 2.7	**0.83** 0.39, 1.8		**1.3** 0.99, 1.6
Spain	2,619	**0.68** 0.49, 0.94	N/A	N/A	N/A			**0.89** 0.49, 1.6	**0.60** 0.49, 0.73	**0.77** 0.63, 0.93	**0.89** 0.75, 1.05	
Sweden [a]	394				**0.86** 0.68, 1.1						**0.05** <0.001, 3.3	**0.47** 0.19, 1.2
Netherlands [a]	1,086											
United Kingdom	599	**1.9** 1.2, 3.2	**2.0** 0.95, 4.1	**1.3** 0.13, 13							**1.7** 0.96, 2.9	
Norway [c]	408										**18** 9.3, 36	**1.6** 1.3, 2.0

[a] Results based on independent logistic regression model.
[b] As the number of samples in some categories is less than 5% of the total number for this country, the significance of the variable should be considered cautiously.
[c] The model for Norway is based on 1 positive slaughter pig only.

- Sampling time – A sine function was used to describe the effect of time of day upon the risk of *Salmonella* infection. A significant association, whatever is the direction, means that there is a difference in the risk of infection between the pigs slaughtered at the end of the working day compared to the beginning. In three countries (Bulgaria, Estonia, and Norway), there was evidence of a significant association of this variable with the risk of infection.
- Sampling quarter – Five countries showed a significant association between this factor and detecting *Salmonella* infection. In Cyprus and Luxembourg the risk was higher in October-December 2006 compared to the summer quarter (July-September) of 2007. Conversely, in Greece the risk in the summer quarter of 2007 was greater than that the first two quarters of the study (October-December 2006 and January-March 2007). In Slovakia and Spain, the risk was greater in the summer quarter of 2007 compared to that of April-June 2007, and to those of quarters January-March 2007 and April-June 2007, respectively.
- Time between sampling and testing – This factor was not significantly ($P < 0.05$) associated with the outcome in any country[1].
- Weight of lymph node samples – in six MSs (Cyprus, Denmark, France, Germany, Italy[2], and Poland[2]), increased weight of lymph node samples generally increased the probability of observing *Salmonella* infection (OR > 1.0), while in two MSs (Belgium and Bulgaria[2]), there was a reduced probability of detecting *Salmonella* infection.
- Number of lymph nodes in the sample – the probability of detecting *Salmonella* infection increased significantly as the number of lymph nodes tested increased in the Czech Republic.

4.2. Analysis of factors associated with surface contamination of carcasses with *Salmonella*

4.2.1. Descriptive analysis of factors potentially associated with *Salmonella* contamination

4.2.1.1 Factors related to the sensitivity of the sampling process

- *Time between the date of sampling and testing in the laboratory*

The time between the date of sampling and testing in the laboratory varied among MSs (Figure 14, Annex III – Table III.1) but being mostly one or two days. In general, *Salmonella* was more likely to be detected from the sample when testing started after 2-4 days from sampling, compared to testing on sampling day or on the following day (Figure 15, Annex III – Table III.2). There was no evidence that testing 5 or more days after the sampling altered the probability of detecting *Salmonella*, although there were fewer observations in this period and thus the results are more uncertain. These results are not adjusted for the country effect. As no linear trend was observed, the "time between the date of sampling and testing" variable was categorised into four levels: 0 day, 1 day, 2 days and 3-7 days (Annex III – Tables III.3 and III.4).

[1] However, in Austria and Denmark, a delay of more than 2 days was associated with an increased probability of observing *Salmonella* infection compared to samples that were tested after less than 2 days, at the significance level of 25%. In Denmark, testing 5 or more days after sampling was associated with a reduced risk of *Salmonella* infection appearing. The time between sampling and testing was found to be significantly associated with *Salmonella* detection at the EU level, probably due to higher statistical power because of a greater sample size.

[2] As the number of samples in some categories is less than 5% of the total number for this country, the significance of the variable should be considered cautiously.

Figure 14. Frequency distribution of the time (days) between sampling and testing of carcass swabs, by MS and for the 13-MS group, and by *Salmonella* status

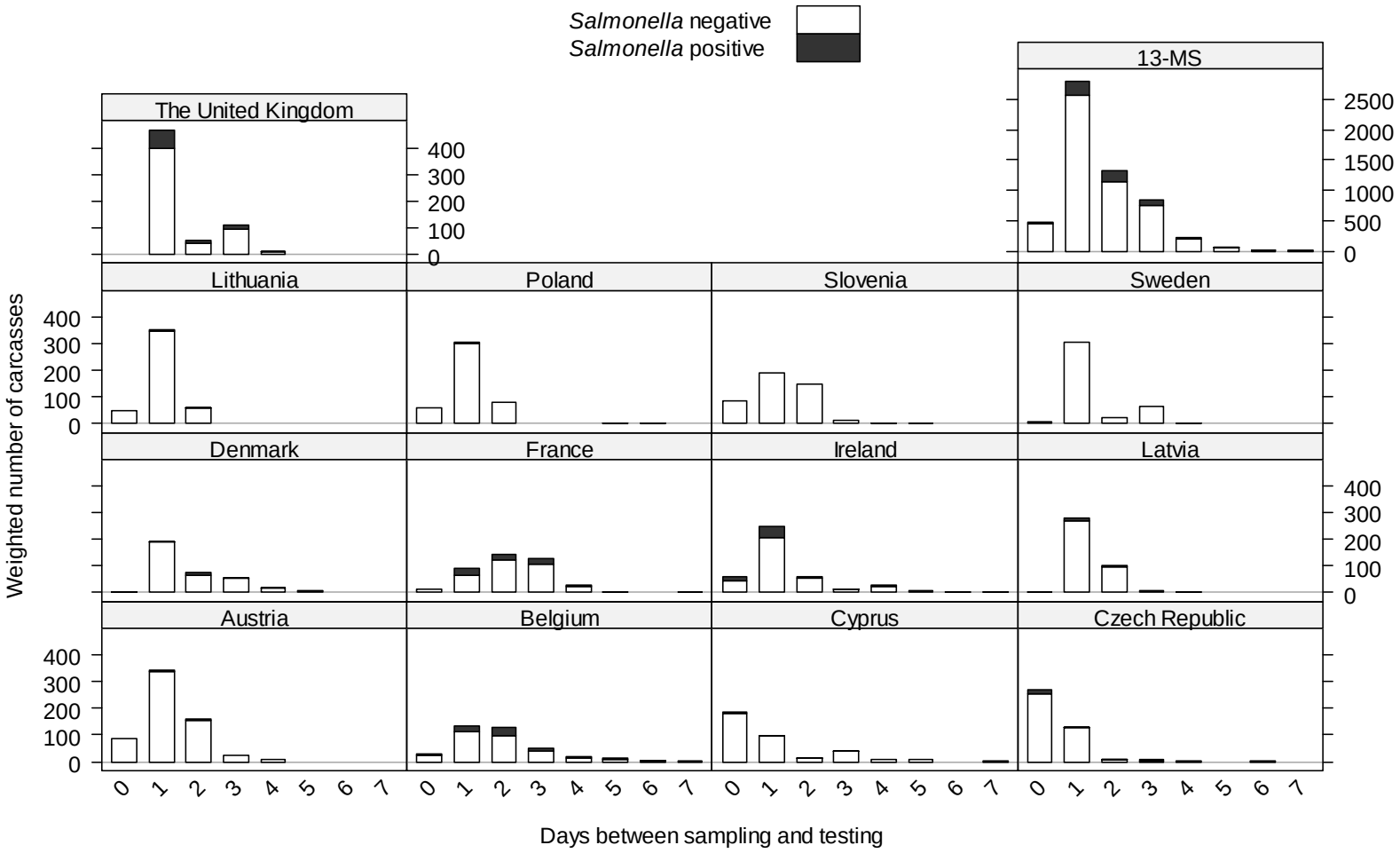
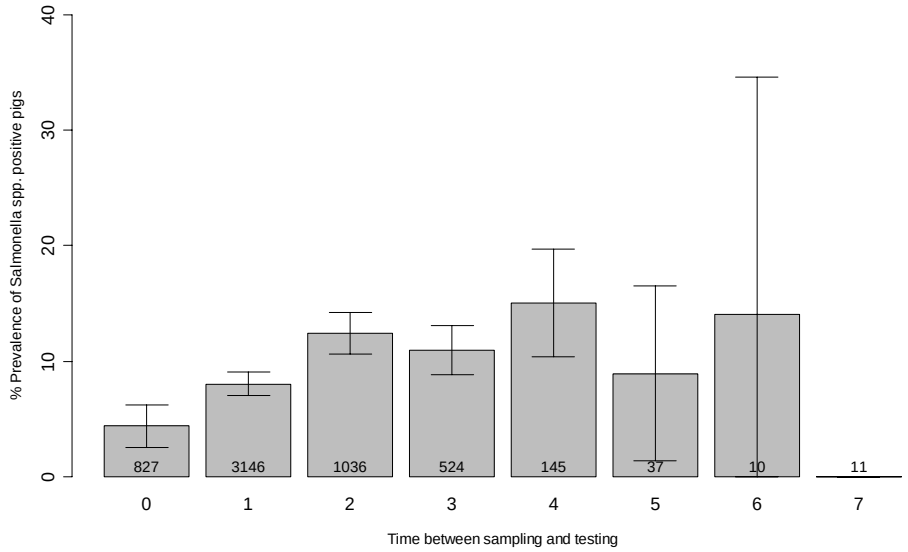


Figure 15. Weighted *Salmonella* prevalence of carcass contamination by number of days between sampling and testing, with 95% confidence intervals, in the 13-MS group

The number of sample pigs is indicated inside each bar.

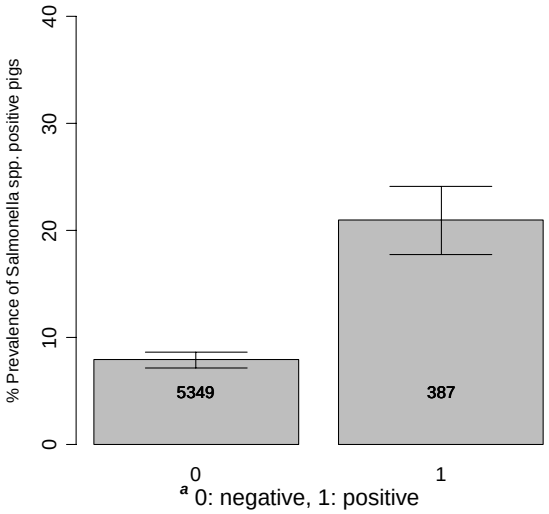


4.2.1.2 Factors related to surface contamination of carcasses

- *Lymph node infection*

The bivariate analysis indicated that there may be an association between the *Salmonella* infection status of slaughter pigs and the *Salmonella* contamination of carcasses, at the 13-MS group level. The weighted prevalence of *Salmonella* contamination of carcasses was greater for slaughter pigs with *Salmonella* infection in lymph nodes compared to the pigs with un-infected lymph nodes (Figure 16).

Figure 16. Weighted prevalence of *Salmonella* contaminated carcasses by *Salmonella* infection status of the slaughter pig, with 95% confidence intervals, in the 13-MS group [a]



- *Analysis of the association between proportions of Salmonella contaminated carcasses and of pigs positive in lymph node samples at the slaughterhouses (146 slaughterhouses where at least 10 pigs were sampled)*

In Figure 17, the proportion of *Salmonella* positive pigs (in lymph nodes) is represented on the horizontal axis, whereas the difference between proportions of *Salmonella* contaminated carcasses and of positive pigs (lymph nodes) is represented on the vertical axis. Each slaughterhouse is represented by a circle, whose size is proportional to the number of pigs sampled in that slaughterhouse. Circles above the horizontal line represent slaughterhouses where the proportion of contaminated carcasses was greater than that of positive pigs, whereas circles below the line correspond to slaughterhouses where proportion of contaminated carcasses was smaller than the proportion of positive pigs. The proportions of *Salmonella* contaminated carcasses and of *Salmonella* infected pigs varied importantly among slaughterhouses. Moreover, the proportion of contaminated carcasses differed importantly between slaughterhouses having a similar proportion of *Salmonella* infected pigs. For example, in the graph, slaughterhouses SH1 and SH2 have both a proportion of *Salmonella* infected pigs of 13.4%, whereas their respective proportion of *Salmonella* contaminated carcasses were 25.4% and 0.1%. For the majority of slaughterhouses, the proportion of contaminated carcasses is lower than the proportion of positive pigs. However, for 35 slaughterhouses (24%) contamination of carcasses is greater than that of pigs.

Figure 17. Bland-Altman graph of the slaughterhouse-specific proportion of *Salmonella* positive pigs against the slaughterhouse-specific difference between proportions of *Salmonella* contaminated carcasses and of *Salmonella* positive pigs

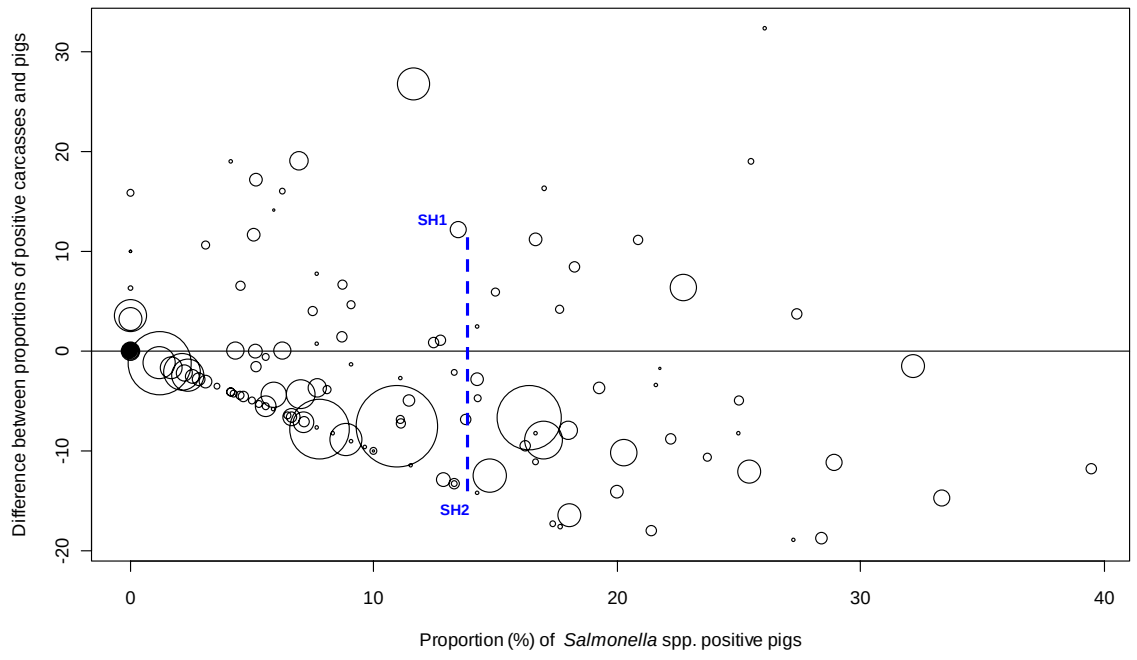
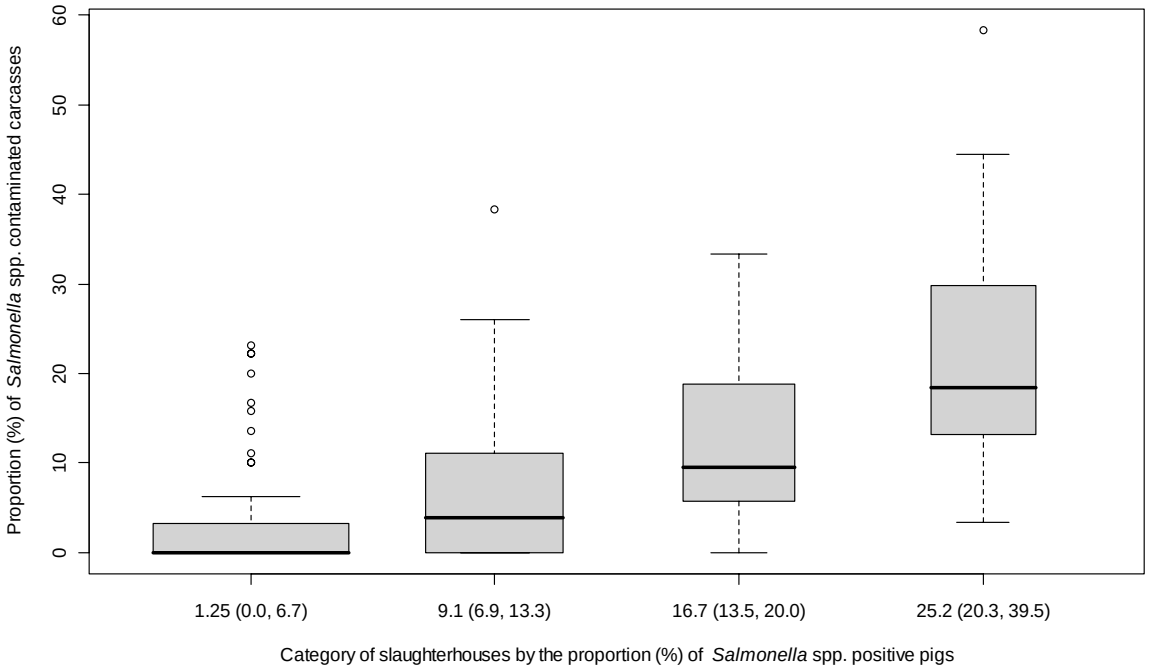


Figure 18 presents a box plot of the proportions of *Salmonella* contaminated carcasses in the slaughterhouses (n=146) categorised by the proportion of *Salmonella* positive pigs. The widths of the box plots are proportional to the number of slaughterhouses per category.

Figure 18. Box plot of slaughterhouse-specific proportion *Salmonella* contaminated carcasses, by slaughterhouse-specific proportion *Salmonella* positive pigs (146 slaughterhouses)



The median (minimum, maximum) of the proportion of positive pigs in each category is reported under the box.

The median proportion of contaminated carcasses (the horizontal line in boxes) and the interquartile range (the height of boxes) appear to increase with the proportion of positive sampled pigs in the slaughterhouse. For a given category (i.e. for slaughterhouses exposed to a comparable ingress of *Salmonella* infected pigs), the proportion of contaminated carcasses varies importantly between the slaughterhouses and this variation is higher for slaughterhouses in the categories having higher proportion of positive pigs.

- *Month of sampling*

A graphical display of the numbers of carcasses sampled at the MS-specific and at the 13-MS group level in each month during the survey is presented in Figure 19 (Annex III – Tables III.5). Sampling of carcasses of slaughter pigs was homogeneous during the survey for most participating countries, although the number of carcasses tested increased progressively during the first four months of the survey. The start of sampling was delayed in Latvia and Lithuania. Generally, *Salmonella* prevalence on carcasses appeared to be lowest at the beginning of the survey (Figure 20, Annex III – Table III.6).

Figure 19. Bar plot of the weighted number of carcass swabs collected by month and MS, and for the 13-MS group, by *Salmonella* status

Months are ordered from October 2006 to September 2007.

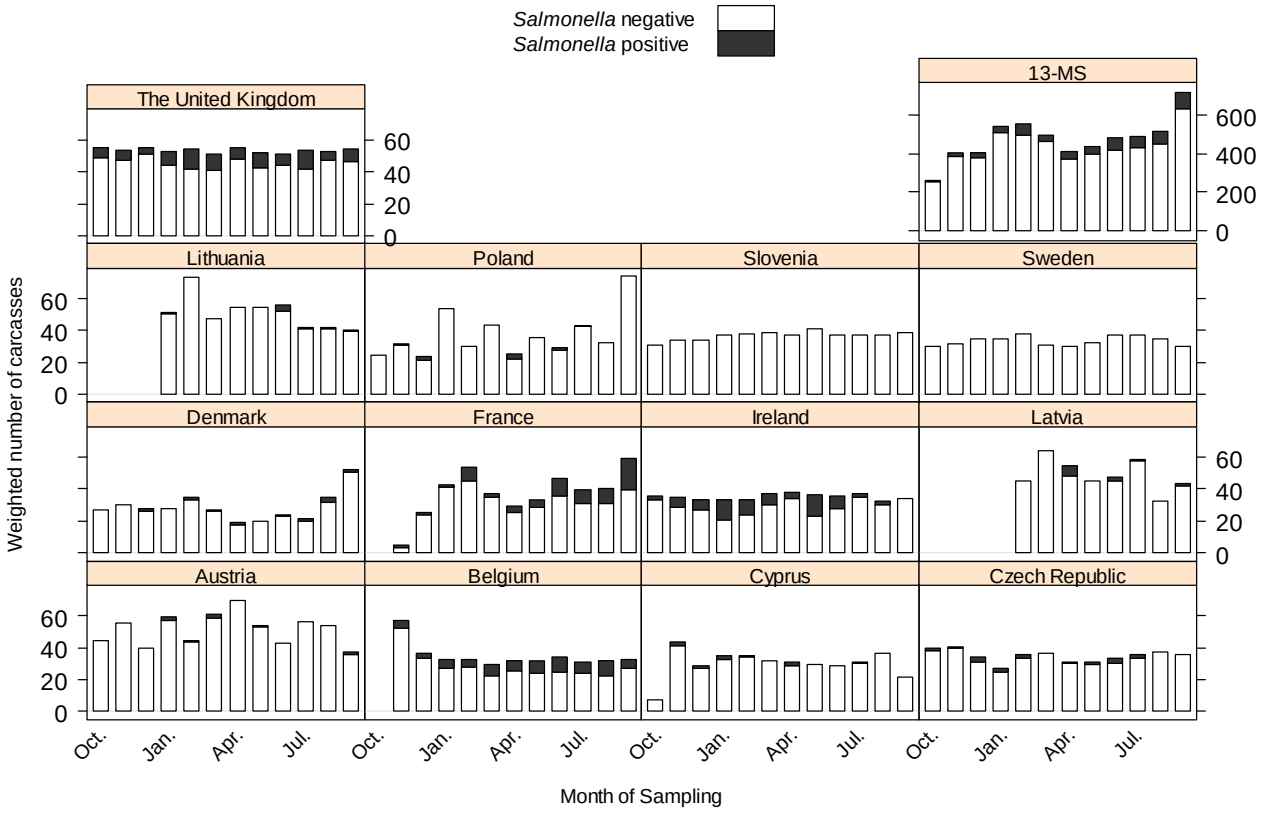
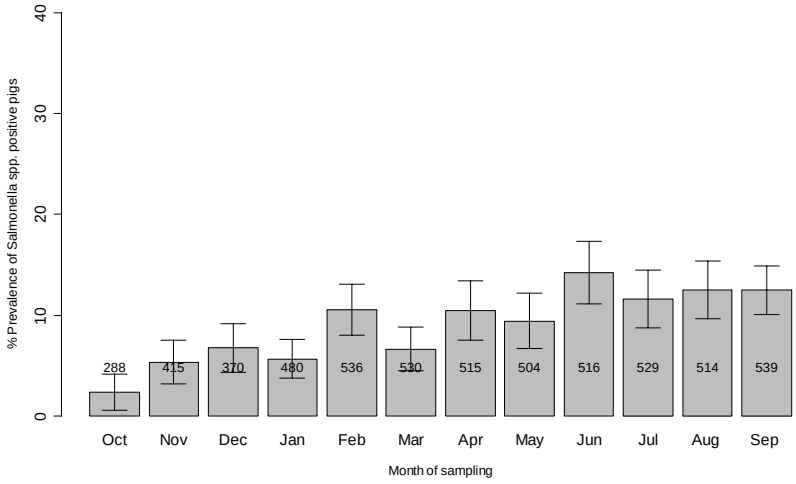


Figure 20. Weighted *Salmonella* prevalence of carcass contamination by month of sampling, with 95% confidence interval, in the 13-MS group

Number of sampled pigs indicated inside each bar.



- *Sampling quarter*

The numbers of carcasses sampled at the MS-specific and at the EU level in each quarter during the survey is presented in Figure 21 (see also Annex III – Table III.7). Some variation in the number of samples was obvious between the quarters of the survey. Generally, the *Salmonella* prevalence on the carcasses appears to increase with the quarter during the survey (Figure 22, see also Annex III – Table III.8).

Figure 21. Bar plot of the weighted number of carcass swabs collected by quarter and MS, and for the 13-MS group, and by *Salmonella* status

Quarters are ordered from October-December 2006 (1) to July-September 2007 (4).

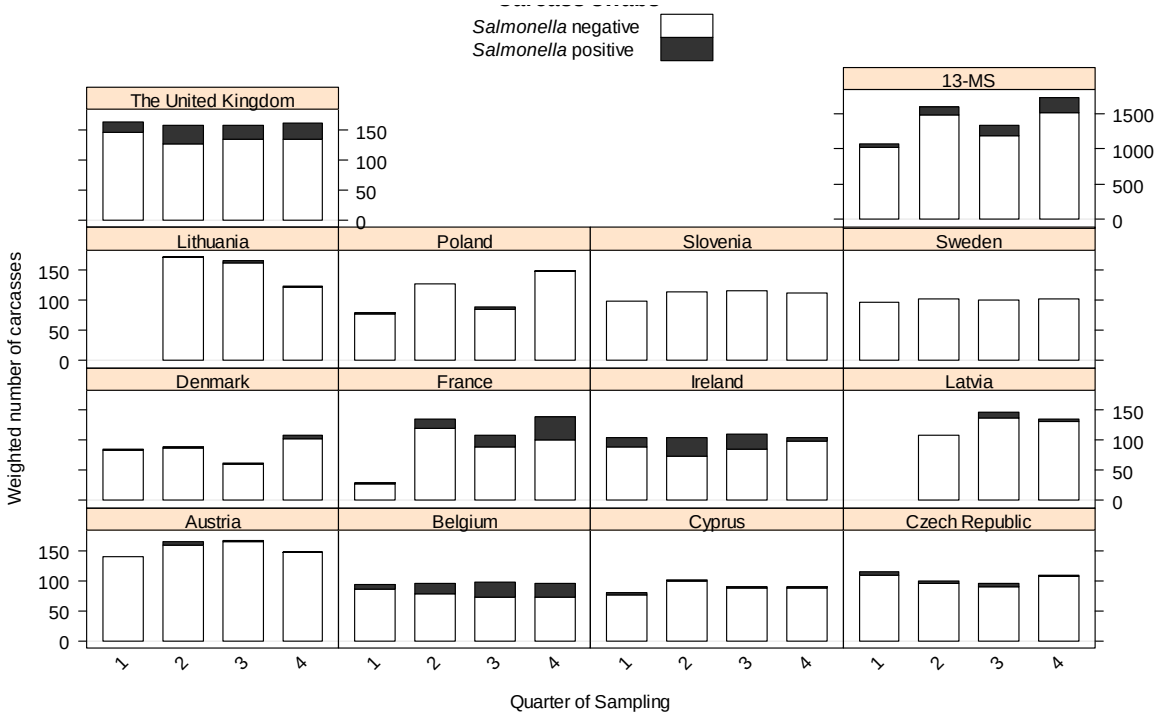
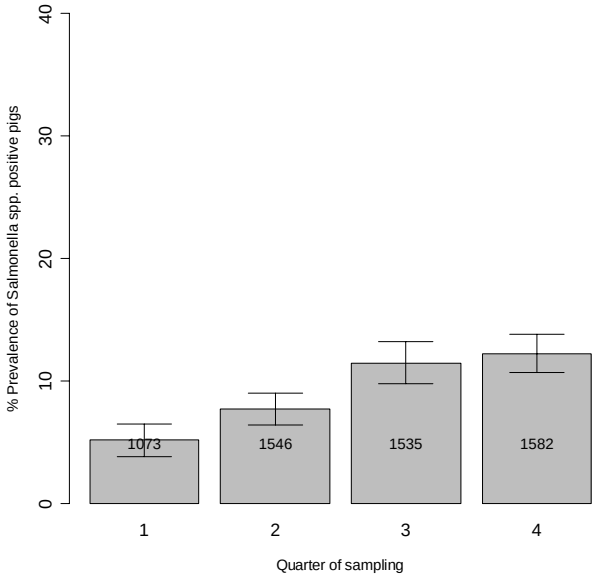


Figure 22. Weighted *Salmonella* prevalence of carcass contamination by sampling quarter, with 95% confidence intervals, in the 13-MS group

Number of sampled pigs are indicated inside each bar.

Quarters are ordered from October-December 2006 (1) to July-September 2007 (4).



- *Hour of sampling*

A graphical display of the number of carcasses sampled at the MS-specific and at the 13-MS group level in each hour of the working day is presented in Figure 23. The results shown in Figure 24 show that there were very few samples taken after 20:00 and before 05:00, thus the seemingly higher prevalence observed during these times must be interpreted with caution (see also Annex III. - Table III.9).

A new variable "Time of sampling (sine)" was created (Annex IV, section IV.1) and used in the building of the model on carcass contamination. A significant effect of this variable would imply that there is a sine trend such that day and night results differ significantly.

Figure 23. Bar plot of the weighted number of carcass swabs collected by hour of sampling and MS, and for the 13-MS group, and by *Salmonella* status

Hours are ordered from 00 to 23.

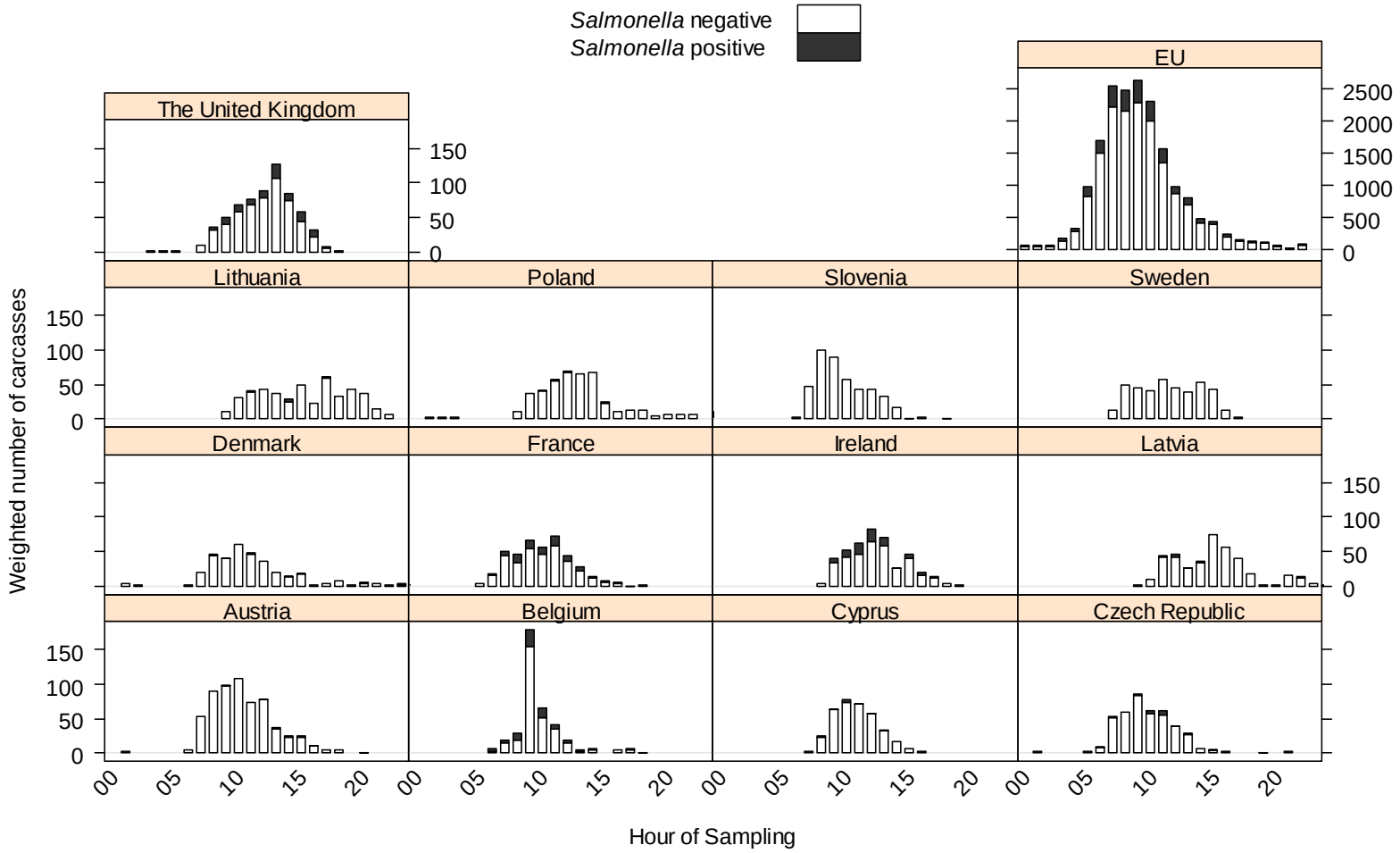
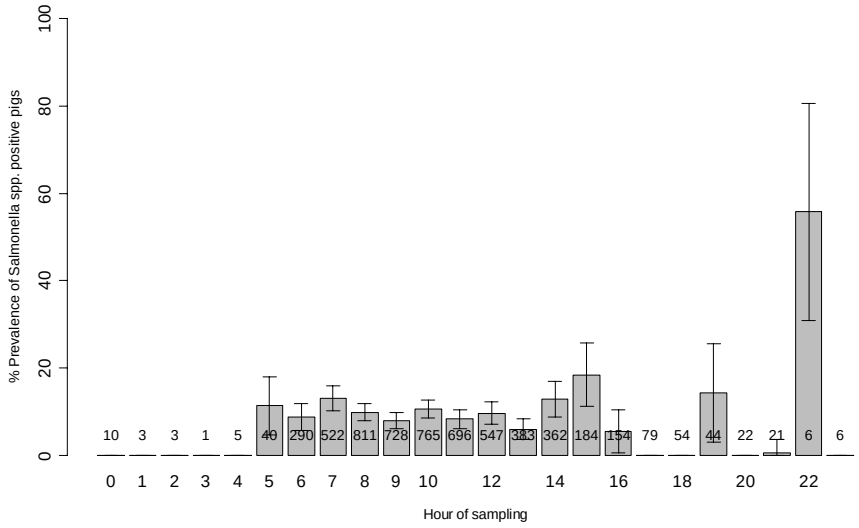


Figure 24. Weighted *Salmonella* prevalence carcass contamination by hour of sampling, with 95% confidence interval, in the 13 MS-group

Number of sampled carcasses indicated inside each bar.



- *Weight of carcasses*

The distribution of carcass weights of slaughter pigs sampled for carcass swabs, at Member State level is shown in Figure 25 (see also Annex III – Table III.10). At the 13-MS group level, the median (Q1; Q3) was 84 kg (76; 92). On average, the heaviest carcasses were sampled in Austria (median=95 kg), whereas the medians were lowest in Cyprus (70 kg), and Latvia (72 kg). The median carcass weight in the group of contaminated carcasses is not different to the median carcass weight of the group of carcasses tested negative for surface contamination (Figure 26 and Annex III – Table III.11).

Figure 25. Box plot of the weight of the carcasses sampled with carcass swabs per MS

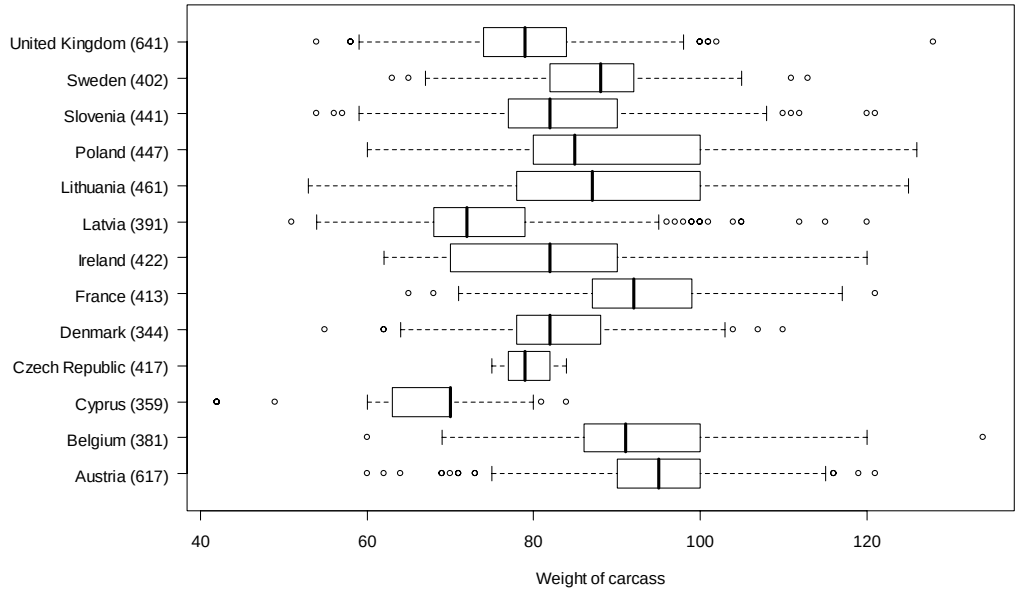
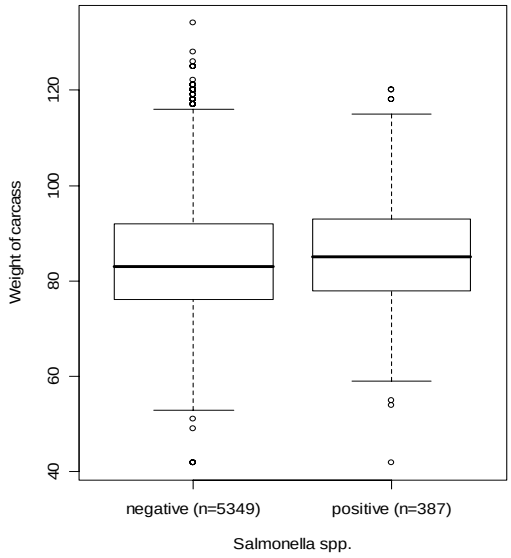


Figure 26. Box plot of the weight of carcasses sampled with carcass swabs, by *Salmonella* status, in the 13-MS group



4.2.2. Analysis of multicollinearity among potential factors

The VIF values calculated for the analysis of multicollinearity among the factors associated with *Salmonella* contamination of carcasses in the 13-MS group are presented in Table IV.3 of Annex IV. This analysis showed that multicollinearity was not an issue for the global model.

The analysis was repeated focusing on each of the participating countries separately and the VIF values are displayed in Table IV.4 of Annex IV. No issues regarding multicollinearity were observed in the analysis carried out by country.

4.2.3. Multiple regression analysis at the 13-MS group level

The factors associated with *Salmonella* surface contamination of carcasses at the 13-MS level are presented in Table 3. The three factors retained in the final model were related to the sensitivity of the sampling and testing process, the infection of slaughter pig lymph nodes and the sampling quarter. The model included significant MS-specific effects (Annex IV, Table IV.7) and therefore, each OR was adjusted for country effect. Correlation among *Salmonella* infection/contamination of pigs/carcasses slaughtered at the same slaughterhouse was taken into account in the model. The slaughterhouse random effects inserted in the model on the intercept and on the slope of the variable "Lymph node infection of the slaughter pigs" were also statistically significant, with *P*-value < 0.0001 and *P*-value = 0.0008, respectively (Annex IV, Table IV.8).

Table 3. The final random effect logistic model for factors associated with the *Salmonella* surface contamination of carcasses of slaughter pigs, in a group of 13 MSs, 2006-2007.

Variables	Random effect logistic model [a, b]	
	OR	95%CI
Time (in days) between the date of sampling and testing in the laboratory [c]		
0 day	0.51	0.28, 0.93
1 day	1	-
2 days	1.009	0.76, 1.3
3 to 7 days	0.70	0.52, 0.96
Lymph node infection of the slaughter pig [c]		
No	1	-
Yes	1.8	1.1, 2.8
Sampling quarter [c]		
Oct. – Dec. 2006	0.51	0.35, 0.72
Jan. – Mar. 2007	0.58	0.44, 0.77
Apr. – Jun. 2007	1.002	0.77, 1.3
Jul. – Sept. 2007	1	-

[a] Estimates and standard errors were assessed using a mixed model with a slaughterhouse random effect on the intercept (*P*-value < 0.0001) and on the slope of the "Lymph node infection of the slaughter pig" variable (*P*-value = 0.0008) and country-specific fixed intercept.

[b] As the country-specific effect of Slovenia and Sweden could not be estimated (no carcass swabs tested positive for *Salmonella*), these countries were not considered in the MS group level analysis.

[c] Significant at *P*-value < 0.05.

According to the analyses, a slaughter pig that was infected by *Salmonella* in the lymph nodes was approximately twice more likely to result in a carcass that is contaminated with *Salmonella* on the surface than a pig whose lymph nodes were not shown to be infected. Also the time of sampling throughout the 1-year survey was shown to have an impact on *Salmonella* contamination of carcasses. The carcasses were less likely to get contaminated in October-December and January-March than in July-September. However, no significant difference was observed between the likelihood of having a *Salmonella* positive carcass in April-June compared to the July-September quarter.

The effect of the time delay between sampling and the start of laboratory testing was identified as a factor associated with the detection of *Salmonella* in carcass swabs. Compared to a test carried out 1-2 days after that of sampling, the likelihood of detecting *Salmonella* is reduced by approximately 50% when the test is performed on the day of sampling and by 30% when delayed by more than 3 days.

The significance of the slaughterhouse random intercept revealed that the baseline risk of *Salmonella* carcass contamination varied between the slaughterhouses, even when other factors such as lymph node infection, month of sampling and time before analysis were accounted for. Moreover, the model indicated that the impact of *Salmonella* infection in pigs, as measured by lymph nodes, on the carcass surface contamination varied between slaughterhouses (significant

slaughterhouse random slope). According to the results, the risk of getting a contaminated carcass from a *Salmonella* infected pig differed between the slaughterhouses. In a similar way, the risk of contaminating a carcass from a pig with *Salmonella* negative lymph nodes depended on the slaughterhouse.

4.2.4. Multiple regression analysis at the MS level

The results of the analysis by country are displayed in Table 4. The different levels of significance are indicated by different shades of grey, darker meaning more significant. Empty cells indicate that the effect of the covariate was not sufficiently significant in that particular country to be maintained in the final model. However, for some multi-level covariates not all categories were available in all countries.

Variability between significant risk factors obtained for each country was observed and some factors even had contrasting effects depending on the country. When these effects are studied at the level of the 13 MS-group, these results may average out so that no significant effect is observed in the 13 MS-group model.

The final models, respectively Austria, Cyprus, Czech Republic, Lithuania, and Poland did not identify any factors of being significantly associated (at a level of 5% or less) with surface contamination of carcasses.

The associations observed for each factor across the MSs are:

- Weight of carcasses (1-kg increments) – This factor was not significantly associated with the outcome in any MS considered.
- Sampling time – A sine function was used to describe the effect of time of day upon the risk of *Salmonella* infection. A significant association, whatever the direction, means that there is a difference in the risk of infection between the pigs slaughtered at the end of the working day compared to the beginning. This factor was not significantly associated with the outcome in any MS.
- Sampling quarter – In three MSs there was a significant association between this factor and detection of *Salmonella* contamination. In Belgium the risk was lower in October-December 2006, whereas it was lower in France in January-March 2007, compared to the summer quarter (July-September) of 2007. Conversely, in Ireland, the risk in January-March 2007 and April-June 2007 was greater than that in the summer quarter of study (July-September 2007).
- Time between sampling and testing – In Denmark, the probability of detecting *Salmonella* in carcass swabs is higher after a delay of 2 days compared to samples tested the day after that of sampling.
- *Salmonella* detection in lymph node samples – In three MSs (France, Latvia, and the United Kingdom), the infection of lymph nodes of the carcass was significantly associated with an increased probability of observing *Salmonella* contamination on the surface of the carcass.

Table 4. Random effect logistic models for factors associated with *Salmonella* surface contamination of carcasses of slaughter pigs for the 13 participating MSs.

Odds ratio estimates and 95%CI are presented for significant (at different levels of significance) risk factors obtained for each country separately. The colour of the cell illustrates the degree of significance (*P*-value) of the association, according to the following scale:

P<0.01: 0.01<*P*<0.05: 0.05<*P*<0.10: 0.10<*P*<0.25:

Country	No. of carcasses	Time between sampling and testing			Lymph node infection	Quarter			Sampling time (sine)	Carcass weight (by 10-kg increments)
		0 day vs. 1 day	2 days vs. 1 day	3 to 7 days vs. 1 day	Yes vs. No	Oct.-Dec.06 vs. Jul.-Sep.07	Jan.-Mar. 07 vs. Jul.-Sep. 07	Apr.-Jun. 07 vs. Jul.-Sep. 07		
Austria	617								**0.06** 0.001, 3.8	
Belgium	381					**0.29** 0.10, 0.81	**0.84** 0.37, 1.9	**1.4** 0.66, 2.9	**2.3** 0.59, 8.9	
Cyprus [a]	359									
Czech Republic	417	**3.1** 0.50, 19	**11** 0.77, 162	**13** 0.93, 170						
Denmark	344	**0.14** <0.01, 999	**18** 2.6, 124	**4.3** 0.52, 36	**3.3** 0.64, 17					**0.93** 0.85, 1.02
France	413	**<0.01** <0.01, -	**0.45** 0.20, 0.97	**0.43** 0.20, 0.93	**2.6** 1.3, 5.1	**0.36** 0.090, 1.4	**0.29** 0.14, 0.60	**0.61** 0.30, 1.2	**0.5** 0.18, 1.3	
Ireland	422	**1.4** 0.69, 2.9	**0.33** 0.12, 0.94	**0.57** 0.21, 1.5		**2.0** 0.72, 5.8	**5.8** 2.2, 15	**3.6** 1.4, 9.8		
Latvia	391				**5.6** 1.2, 26					
Lithuania	461					N/A			**0.04** 0.00, 1.32	
Poland	447									
United Kingdom	641				**2.3** 1.4, 3.8					

[a] Results are based on independent logistic regression.

4.3. Analysis of the serovars and phage type distribution

4.3.1. Spatial distribution of *Salmonella* serovars in lymph nodes

To investigate the spatial distribution of the most frequently reported serovars isolated from the lymph nodes of slaughter pigs, the scan statistic was performed. Bulgaria was not included in the analysis due to the lack of data on their actual production numbers. Table 5 shows the most likely spatial clusters with their respective relative risk (RR) and level of significance (*P*-value).

Table 5. Most likely clusters of *Salmonella* spp., *S.* Typhimurium, *S.* Derby, *S.* Enteritidis, *S.* Infantis, and *S.* Rissen in MSs

Serovar	Most Likely Cluster	Relative Risk[a]	Secondary high risk MSs	Relative Risk[a]
Salmonella spp.	PT, ES, IE, FR, UK, LU	2.6	GR	1.8
			IT	1.2
S. Typhimurium	PT, ES, IE, FR, UK, LU, BE	2.5	-	-
S. Derby	PT, ES, IE, FR, UK, LU, BE, NL, IT	3.9	GR	1.3
S. Enteritidis	HU, SK, SI, CZ, PL	5.1	PT	3.0
S. Infantis	DK, DE	3.6	FR	2.1
S. Rissen	PT, ES	201.4	-	-

[a] *P*-value = 0.001

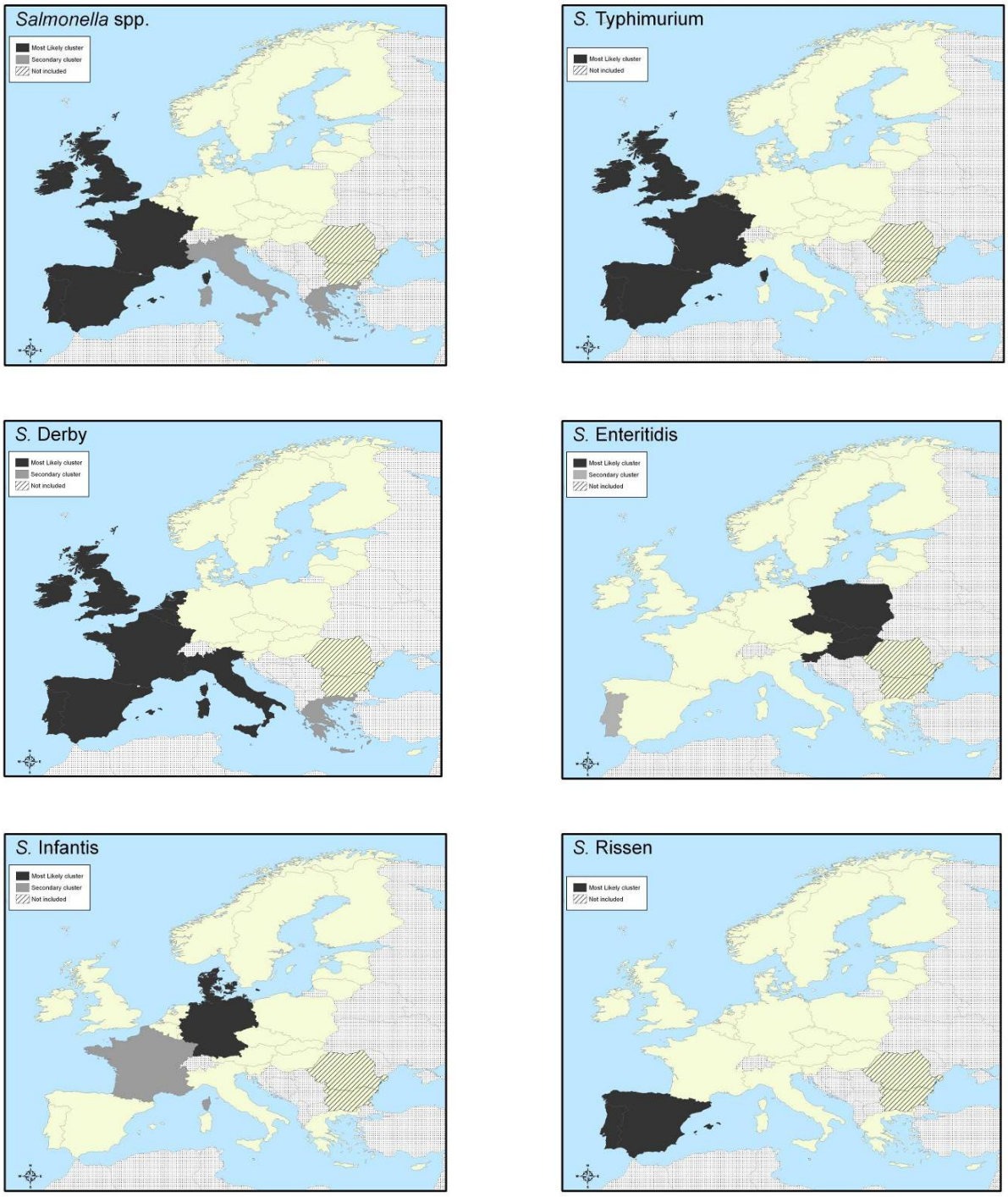
Among slaughter pigs (ileo-caecal lymph node samples), spatial cluster analysis showed that the most likely cluster for *Salmonella* included six countries (Portugal, Spain, Ireland, France, the United Kingdom, Luxembourg). A significant RR of 2.6 suggested that slaughter pigs in these countries are 2.6 times more likely to become infected than slaughter pigs outside those countries. After detection of most likely clusters, the scan statistic also identified, for some serovars, other single MSs (e.g. Greece and Italy for *Salmonella*) with a risk of *Salmonella* infection in slaughter pigs significantly above the EU average but lower than the risk in the most likely clusters of MSs.

The most likely spatial cluster for *S.* Typhimurium included Portugal, Spain, Ireland, France, the United Kingdom, Luxembourg, and Belgium. This cluster of countries presented a relatively high RR for this serovar (RR = 2.5). A similar scenario was found for *S.* Derby, but this cluster presented a larger radius and included also the Netherlands and Italy. *S.* Enteritidis clustered spatially in Eastern Europe, whereas *S.* Infantis is clustered in Denmark and Germany. Finally, Portugal and Spain, which were detected as the most likely cluster for *S.* Rissen, were also included in the most likely clusters for three other of the proposed spatial scan analyses.

Maps of most likely and secondary clusters presented in Table 5 are displayed in Figure 27.

Figure 27. Most likely clusters of *Salmonella* spp., *S.* Typhimurium, *S.* Derby, *S.* Enteritidis, *S.* Infantis, and *S.* Rissen in slaughter pigs (ileo-caecal lymph nodes)

No data available from Bulgaria, Romania and Malta.

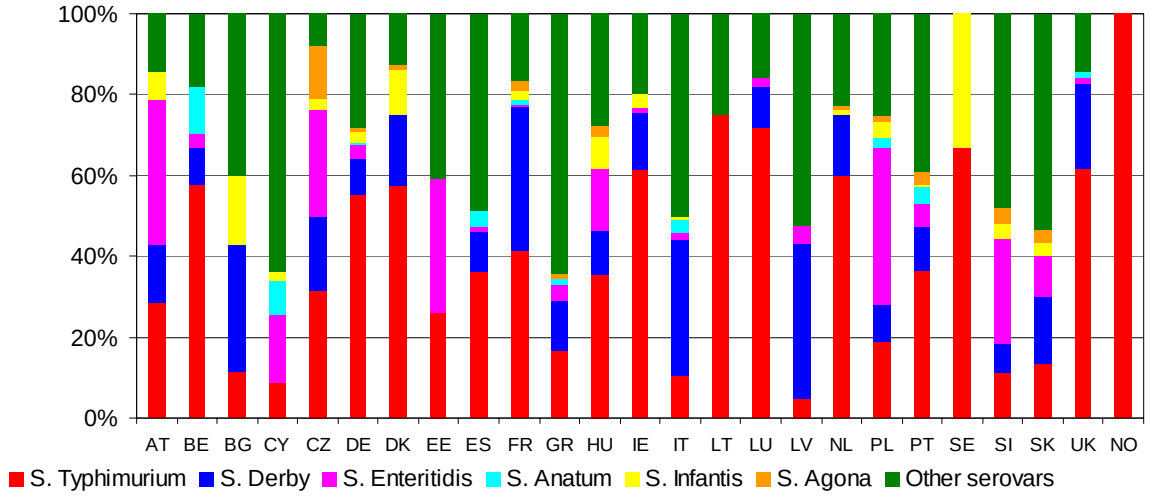


4.3.2. Differences in serovar distribution between the reporting countries

As reported in the Part A report, the diversity of isolated serovars in the ileo-caecal lymph node samples differed considerably between MSs from two serovars in Sweden and Lithuania up to more than 20 serovars in Germany, France, Greece and Spain. The diversity was particularly pronounced in Greece, where only 345 pigs were sampled, whereas the sample sizes were three to seven times larger in the three other MSs mentioned.

S. Typhimurium and *S.* Derby are widespread and dominant in slaughter pigs across most of the MSs (Figure 28). However, *S.* Enteritidis had a relatively high prevalence in eight MSs.

Figure 28. Relative frequency distribution of *S.* Typhimurium, *S.* Derby, *S.* Enteritidis, *S.* Anatum, *S.* Infantis, *S.* Agona and other *Salmonella* serovars isolated in MSs from ileo-caecal lymph nodes from slaughter pigs, 2006-2007.



4.3.3. Comparison between serovar distributions in slaughter pigs, the animal species, feed and human cases in the EU

Salmonella Enteritidis, the most frequent cause of human salmonellosis, was relatively rare in slaughter pigs. Therefore, it is excluded from this visual analysis to allow an effective comparison of frequencies of other serovars. *S.* Enteritidis in humans is broadly recognised to be principally associated with the poultry food chains, particularly the consumption of table eggs and products thereof (EFSA, 2007). For the same purpose, *S.* Rissen and *S.* 4,[5],12:i: were removed from the pig data for comparison because these serovars highly clustered in Spain and Portugal (which both accounted respectively for 98% and 89% of the lymph node isolates of these serovars) and were not reported as associated with human cases of salmonellosis in Spain (data not available for Portugal).

Disregarding the contribution of *S.* Enteritidis, *S.* Rissen and *S.* 4,[5],12:i:, Figure 29 and Figure 30 respectively compare relative *Salmonella* serovar distribution of the most frequent serovars

isolated from slaughter pigs in lymph node samples as well as carcass swabs with that of serovars reported in human salmonellosis cases. There were only minor differences between the most frequently isolated serovars in lymph nodes as compared to carcasses. Moreover, disregarding *S.* Enteritidis, there appears to be some agreement between the most frequently reported serovars in humans and those isolated in slaughter pigs. This is particularly the case for isolates from the carcass surface. However, some discrepancies were also observed. For example, countries where *S.* Derby was relatively dominant in carcasses did not demonstrate analogous proportions in humans (e.g. Austria, Latvia). Conversely, some countries could report a significant serovar in humans and did not observe analogous findings in carcasses (e.g. *S.* Infantis in Austria and the United Kingdom or *S.* Derby in Cyprus).

Figure 29. Comparison of the *Salmonella* serovar distribution in ileo-caecal lymph nodes from slaughter pigs and humans (TESSy, 2006) in MSs and Norway.

Only the distribution of the most commonly isolated serovars in slaughter pigs is presented. *S.* Enteritidis, *S.* Rissen, and *S.* 4,[5],12:i:- were excluded from this figure.

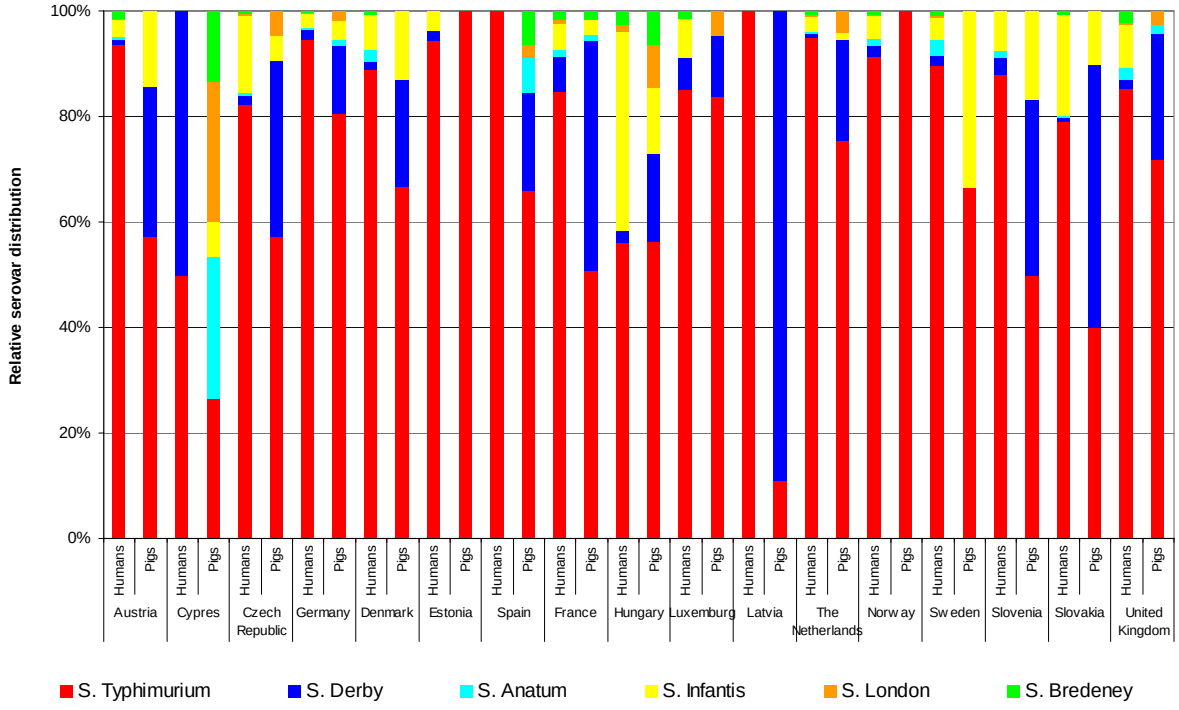
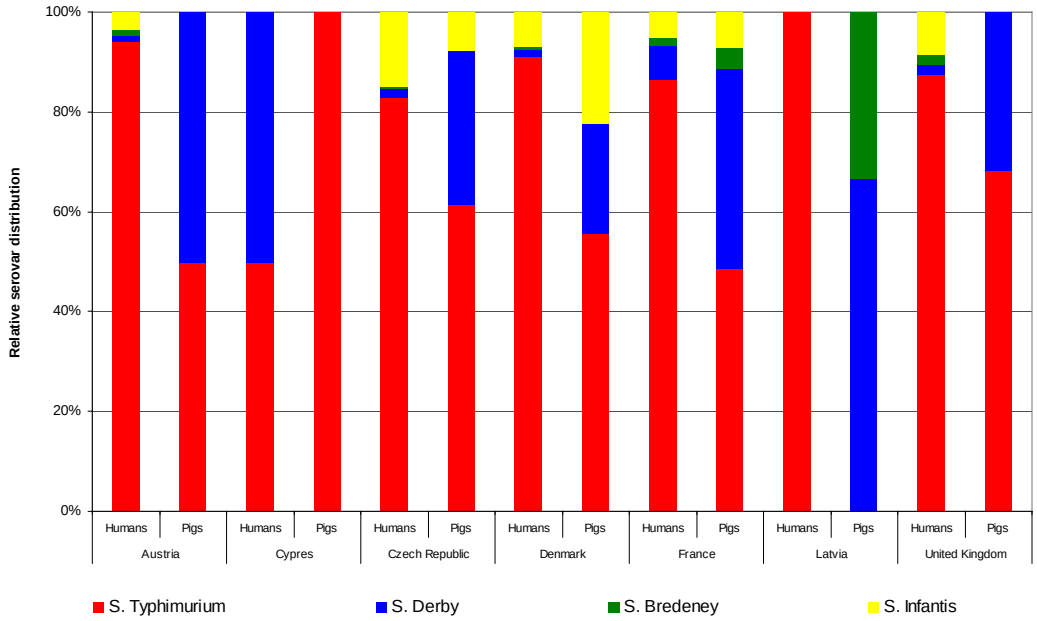


Figure 30. Comparison of the *Salmonella* serovar distribution in carcass swabs from slaughter pigs and humans (TESSy, 2006) in seven MSs.

Only the distribution of the most commonly isolated serovars in slaughter pigs is presented. *S.* Enteritidis was excluded from this figure.



The comparison of the distribution of serovars isolated from feed and ileo-caecal lymph node samples of slaughter pigs showed that many of the serovars detected in slaughter pigs are also isolated from feed (Table 6). Serovars isolated from slaughter pigs have also been isolated from broilers and laying hens (e.g. *S.* Infantis and *S.* Livingstone), and *S.* Derby, *S.* Bredeney and *S.* London are shared with turkeys. In contrast, *S.* Rissen, *S.* Bovismorfibicans, *S.* Goldcoast, *S.* Give, and *S.* Thompson were only isolated from slaughter pigs.

Table 6. Number of isolations of *Salmonella* serovars from slaughter pigs (baseline survey 2006-2007), pig feed (2006), humans (ECDC), broiler flocks (baseline survey 2005-2006), laying hen holdings (baseline survey 2004-2005), and turkey flocks (baseline survey 2006-2007).

Salmonella serovars	Slaughter pigs	Humans	Feed (pigs, oil seed and fruit)	Broilers flocks	Laying hens holdings	Turkeys flocks
S. Typhimurium	1,040	19,009	Yes	65	123	86
S. Derby	380	484	Yes	13	14	123
S. Rissen	151	105	Yes	-	-	-
S. Enteritidis	126	91,325	Yes	538	899	55
S. Anatum	63	159	Yes	32	21	-
S. Bredeney	51	160	Yes	10	26	186
S. Infantis	49	1,261	Yes	295	171	72
S. London	33	88	-	-	-	31
S. Brandenburg	31	243	-	-	-	-
S. Agona	28	388	Yes	16	38	31
S. Newport	24	751	Yes	8	11	33
S. Montevideo	19	359	Yes	31	27	13
S. Bovismorbificans	15	304	-	-	-	-
S. Goldcoast	14	143	-	-	-	-
S. Give	11	191	Yes	-	-	-
S. Livingstone	9	86	Yes	39	50	-
S. Thompson	9	196	Yes	-	-	-
S. Hadar	8	726	-	59	53	152

4.3.4. Phage type distributions

4.3.4.1 *S.* Enteritidis phage types

Data on *S.* Enteritidis phage types were only provided from the ileo-caecal lymph node isolates from slaughter pigs by four MSs (Austria, Belgium, Hungary and the United Kingdom). Fifteen MSs with *S.* Enteritidis isolates did not report phage typing information. Only one out of three MSs (Austria) phage typed a *S.* Enteritidis isolate from the carcass swabs.

The four MSs providing information on *S.* Enteritidis phage types from lymph nodes reported a total of 22 isolates out of which 17 (77%) were phage typed. This represented 14% of all 126 reported *S.* Enteritidis isolates from ileo-caecal lymph nodes of slaughter pigs in the EU baseline survey. Reported phage types from lymph node and carcass swab samples are presented in Table 7. In this table the ranking is based on the number of specific *S.* Enteritidis phage type-positive slaughter pigs in the four MSs.

Table 7. Distribution of the *S.* Enteritidis phage types in slaughter pigs in four reporting MSs, EU slaughter pigs baseline survey, 2006-2007.

Phage type	Ileo-caecal lymph nodes		Carcass-swabs	
	No. of strains	MSs reporting phage types	No. of strains	MS reporting phage types
PT 8	5	AT, HU	-	-
PT 4	3	AT, HU	1	AT
PT 1	1	AT	-	-
PT 4b	1	UK	-	-
PT 5a	1	AT	-	-
PT 6a	1	AT	-	-
PT 9a	1	BE	-	-
PT 13	1	HU	-	-
PT 13a	1	UK	-	-
PT 20	1	BE	-	-
PT 21	1	BE	-	-

4.3.4.2 *S.* Typhimurium phage types

Data on *S.* Typhimurium phage types was provided from ileo-caecal lymph node isolates by five MSs (Belgium, Hungary, Sweden, the Netherlands and the United Kingdom), whereas the remaining 19 MSs with *S.* Typhimurium isolates did not provide any phage typing information.

The MSs that reported information regarding *S.* Typhimurium phage types from lymph nodes reported 217 isolates out of which 207 (95%) were phage typed. This represented 20% of the all 1,040 reported *S.* Typhimurium isolates in the EU baseline survey. The reported phage types in Belgium, Hungary, Sweden, and the United Kingdom are presented in Table 8. The ranking is based on the percentages of *S.* Typhimurium phage type-positive ileo-caecal lymph node isolates in those four MSs. The results reported by the Netherlands are presented separately in Table 9, because of a different phage typing system used in this country.

Phage type information on carcass swab isolates was reported from two (Belgium and the United Kingdom) of the 13 MSs having undertaken the carcass swab study. A total of 80 out of 93 isolates (86%) in the two MSs was phage typed. In total, 191 isolates were reported from the ten MSs isolating *S.* Typhimurium from the carcass swabs.

A total of 23 phage types (excluding RDNC) were recorded in lymph node samples and 13 in carcass swabs, indicating a large diversity of *S.* Typhimurium phage types in pigs. There was some overlap of phage types in lymph nodes and carcass swabs, but 10 phage types were only isolated from lymph node samples, whereas four phage types were only found in carcass swabs. Phage type U288 was the most frequently reported one but reported only in the United Kingdom. DT 193 was the second most frequently recorded phage type and found in Belgium, Hungary, and the United Kingdom in both lymph nodes and carcass swabs. FT 506 and 507 accounted for more than half the *S.* Typhimurium phage types in the Netherlands. However, one third of the isolates were non typeable.

Table 8. Distribution of the *S.* Typhimurium phage types in slaughter pigs in four reporting MSs, EU slaughter pigs baseline survey, 2006-2007.

	Ileo-caecal lymph nodes		Carcass-swabs	
Phage type	**No. of strains**	**MSs reporting phage types**	**No. of strains**	**MSs reporting phage types**
U 288	28	UK	16	UK
DT 193	26	BE, HU, UK	13	BE, UK
U 302	19	BE, HU, UK	2	UK
DT 104	16	BE, HU, UK	4	BE, UK
DT 104b	8	UK	3	UK
DT 120	7	BE, HU	13	BE, UK
DT 12	4	BE	2	BE
DT 135	3	HU	-	-
DT 40	2	SE	-	-
DT 56	2	UK	-	-
DT 193w	2	BE	1	BE
DT 208	2	UK	4	BE, UK
DT 2	1	BE	-	-
DT 41	1	SE	-	-
DT 56a	1	UK	-	-
DT 85	1	UK	-	-
DT 194	1	UK	-	-
U 277	1	SE	-	-
U 310	1	BE	-	-
DT 110	-	-	2	BE
DT 185	-	-	2	BE
DT 35	-	-	1	BE
DT 170	-	-	1	UK
RDNC[a]	17	BE, HU, UK	5	BE, UK
Non typeable	11	BE, HU, UK	11	BE, UK

[a] RDNC: 'Reacts but Does Not Conform'.

Table 9. Distribution of the *S.* Typhimurium phage types in slaughter pigs in the Netherlands, 2006-2007.

	Ileo-caecal lymph nodes
Phage type	**No. of strains**
FT 506	13
FT 507	12
FT 510	3
FT 508	2
FT 2	1
FT 110	1
FT 292	1
FT 301	1
FT 401	1
Non typeable	18

4.3.5. Comparison between phage type distribution in slaughter pigs and humans

In order to further investigate the role of pig meat as a source of human *S*. Enteritidis and *S*. Typhimurium infection, the phage typing results from the slaughter pig baseline survey and isolates from humans (Community Summary Report, 2006) were compared (Table 10, 11 and 12). Phage type distributions in humans were only available from a fraction of the MSs and, as reported earlier, only a minor proportion of the MSs reported the phage types of the isolates found in the baseline surveys. Interpretation should consequently be carried out cautiously due to limited numbers and lack of representativeness. Some phage types were shared by the human cases and pigs in the reporting MSs.

Table 10. Comparison of *S.* Enteritidis phage types isolated from humans and slaughter pigs (ileo-caecal lymph nodes and carcass swabs).

	No. of human *S.* Enteritidis phage types reported in 2006 [a]							No. of slaughter pigs as reported in the EU baseline survey, 2006-2007			
Phage type	**AT**	**CZ**	**HU**	**NL**	**PT**	**UK**	**Total**	**AT**	**BE**	**HU**	**UK**
PT 4	1,125	3	398	315	-	2,069	3,910	2	-	2	-
PT 8	964	90	642	41	-	1,088	2,825	1	-	4	-
PT 1	212	4	22	47	-	1,492	1,777	1	-	-	-
PT 21	884	2	174	55	-	609	1,724	-	1	-	-
PT 6	371	1	246	69	-	246	933	-	-	-	-
PT 14b	67	-	20	9	23	538	657	-	-	-	-
PT 6a	201	-	-	17	-	218	436	1	-	-	-
PT 1b	3	1	85	-	296	12	397	-	-	-	-
PT 13a	30	13	113	-	-	117	273	-	-	-	1
RDNC	91	-	89	-	-	46	226	-	-	-	-
PT 13	1	83	44	-	-	1	129	-	-	1	-
PT 56	-	-	-	-	-	93	93	-	-	-	-
PT 11	3	-	-	8	-	78	89	-	-	-	-
PT 3	38	-	-	10	-	14	62	-	-	-	-
PT 1c	56	-	-	-	-	2	58	-	-	-	-
PT 4b	5	6	22	2	28	4	57	-	-	-	1
PT 2	11	-	32	1	-	2	46	-	-	-	-
PT 23	10	4	20	2	-	-	36	-	-	-	-
PT 7	33	-	-	2	-	-	35	-	-	-	-
PT U	32	-	-	-	-	-	32	-	-	-	-
PT 19	27	-	-	-	-	-	27	-	-	-	-
PT 6c	-	-	24	-	-	-	24	-	-	-	-
Not typeable	-	-	28	-	23	20	71	-	-	-	-
Other	79	6	59	15	47	1,089	1,295	1	2	-	-

[a] Source: European Centre for Disease prevention and Control (ECDC).

Table 11. Comparison of *S.* Typhimurium phage types isolated from humans and slaughter pigs (ileo-caecal lymph nodes and carcass swabs).

	No. of human *S.* Typhimurium phage types reported in 2006 [a]					No. of slaughter pigs as reported in the EU baseline survey, 2006-2007					
						Ileo-caecal lymph nodes				Carcass swabs	
Phage type	**AT**	**CZ**	**HU**	**UK**	**Total**	**BE**	**HU**	**SE**	**UK**	**BE**	**UK**
DT 104	-	63	-	370	433	3	8	-	5	3	1
DT 46	267	-	-	-	267	-	-	-	-	-	-
DT 193	14	-	62	108	184	5	1	-	20	2	11
DT 104l	79	-	103	-	182	-	-	-	-	-	-
RDNC	92	-	24	46	162	12	3	-	2	-	-
DT 104b	-	-	64	72	136	-	-	-	8	-	3
DT 120	33	8	-	73	114	4	3	-	-	12	1
DT 8	4	-	-	93	97	-	-	-	-	-	-
DT 1	18	22	-	46	86	-	-	-	-	-	-
DT 41	68	3	-	9	80	-	-	1	-	-	-
U 302	-	-	45	10	55	2	8	-	9	-	2
DT 56	-	-	-	50	50	-	-	-	2	-	-
DT 135	-	2	-	44	46	-	3	-	-	-	-
U 311	-	-	-	38	38	-	-	-	-	-	-
U 288	-	-	-	37	37	-	-	-	28	-	16
DT U	18	5	-	-	23	-	-	-		-	-
DT 208	-	-	14	1	15	-	-	-	2	1	3
DT 35	-	-	14	-	14	-	-	-	-	1	-
DT 194	-	14	-	-	14	-	-	-	1	-	-
DT 125	-	-	13	-	13	-	-	-	-	-	-
Not typeable	-	-	33	13	46	3	1	-	7	3	8
Other	34	31	60	725	850	8	-	3	2	11	2

[a] Source: European Centre for Disease prevention and Control (ECDC).

Table 12. Comparison of *S.* Typhimurium phage types isolated from humans and slaughter pigs (ileo-caecal lymph nodes and carcass swabs) in the Netherlands.

Phage type	No. of human *S.* Typhimurium phage types reported from NL in 2006	No. of slaughter pigs as reported from NL in the EU baseline survey, 2006-2007
FT 561	185	
FT 507	116	12
FT 506	79	13
FT 510	27	3
FT 296	21	
FT 401	8	
FT 508	7	2
FT 60	7	
FT 3	6	
FT 61	4	
FT 80	4	
FT 2	2	1
other	39	4

5. Discussion

Salmonella infections in pigs are often sub-clinical, although some animals may show clinical signs varying from mild diarrhoea to acute septicaemia and death. There is convincing evidence that some human cases of salmonellosis may derive from *Salmonella* infection of pigs or contaminated pig products although the population attributable fraction has not been estimated (EFSA, 2006b). The main motivation to control *Salmonella* infection in pigs is to protect public health.

5.1. Analysis of factors associated with *Salmonella* infection in lymph nodes or surface contamination of carcasses

This EU-wide baseline survey estimated the prevalence of fattening pigs infected with *Salmonella* at slaughter within the 27 Member States (MSs) and these estimates were published in the Part A report. In addition, 13 MSs sampled carcass surfaces and the prevalence of *Salmonella* positive carcass swabs was also reported in Part A. During the conduct of the survey, some compulsory complementary data was recorded. This Part B report considers whether any of these factors were associated with the risk of isolation of *Salmonella* from either the ileo-caecal lymph nodes or from the surface of carcasses. It should be noted that many potential factors of relevance to *Salmonella* infection in slaughter pigs or surface contamination of carcasses such as - among others - hygiene during slaughter and subsequent processing, slaughter techniques, the speed of the slaughter line and the cleaning and disinfection procedures used, were not part of the present survey. 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.

In the EU level multiple regression analyses, the structure of the statistical models took into account the fact that slaughter pigs originating from the same country have a higher probability of sharing similar domestic conditions, that pigs slaughtered in the same slaughterhouse were more likely to have comparable rearing, transport and lairage conditions, and that carcasses from the same slaughterhouse were submitted to similar processes. Furthermore, possible country confounding effects were also taken account of in the analyses.

The analyses performed at MS level in this survey should be regarded as a preliminary attempt to investigate effects of mandatory reported factors in each MS. Moreover, it allowed the assessment of the variability of those effects between MSs. The results of these analyses showed that the importance of the identified factors varied importantly between MSs, as indeed the exposure to some factors was protective in some MSs but increased the risk in others. Consequently, these risk factor analyses results at MS level should be regarded as indicative and need to be complemented by specific studies carried out at national level, taking into account domestic conditions.

5.1.1. Effect of sampling and testing procedures

The multiple regression analyses carried out at the EU level demonstrate that variations in the implementation of sampling and testing procedures had an impact on the probability of detecting *Salmonella* in the samples.

The probability of detecting *Salmonella* from a lymph node sample was highest when the delay between sample collection and laboratory testing was 3-4 days and then decreased once again. A similar increase in the detection rate after 1-2 days from sampling was observed in carcass swab samples. The significant impact of delayed testing on the likelihood of detecting the *Salmonella* has previously been observed in stools of human origin (Poisson et al., 1993).

There might be several biological explanations to this phenomenon, such as a short-term die-off of competing bacteria in the sample to such an extent that *Salmonella* identification is less likely to fail due to the overgrowth of other bacteria in the two to three days following sampling. Subsequently, the decline in numbers of *Salmonella* bacteria, which occurs from the first day, becomes the major factor. It might also be that stress induced by a changed environment due to sampling and subsequent refrigeration of the sample prevented the initial growth of *Salmonella* as the bacterium might have needed to adapt to new conditions.

An increased probability in detecting *Salmonella* from heavier lymph node samples was observed in the survey. This may simply be due to a greater chance of including any *Salmonella* or harvesting a larger number of *Salmonella* bacteria in a heavier sample. A similar effect of the weight of faecal samples on the probability of *Salmonella* detection has been demonstrated previously (Funk et al., 2000; Champagne et al., 2005). However, it might also be that the larger weight of the lymph nodes was in some cases due to an inflammatory reaction to *Salmonella* infection, which would also increase the probability of detecting the bacterium.

These results demonstrate that the applied sampling and testing procedures had an impact on the detection of *Salmonella.* It is possible that the slaughter pig *Salmonella* prevalence estimates presented in the Part A report for MSs might have been partly different if all MSs had applied exactly the same sampling and testing procedures. The results imply that the standardisation of sampling instructions and of testing procedures is important in the forthcoming national *Salmonella* control programmes in pigs. This standardisation would enhance data comparability, at least within the country and also at the EU level.

5.1.2. Effect of lymph node *Salmonella* infection on surface contamination of carcasses

In this survey, *Salmonella* surface contamination of the carcass was affected by the *Salmonella* infection status of the pig as reflected by the lymph nodes. A *Salmonella* infected pig was twice more likely to yield a *Salmonella* contaminated carcass. This can be regarded as an expected finding, as slaughter pig infection and carcass contamination often arise from the same sources, such as intestinal carriage of the pig or contamination from the slaughterhouse lairage environment. Other studies have reported similar associations between the *Salmonella* infection status of the pig before slaughtering and the surface contamination of the carcass (Hald et al., 2003; Sorensen et al., 2004; Mc Dowell, 2007).

Lymph node infection can be considered as a marker of the asymptomatic intestinal carriage of *Salmonella.* The infection can develop on the farm, during transport, or in slaughterhouse lairage. This intestinal *Salmonella* carriage or infection may result in carcass contamination during the slaughter process in the case of faecal leakage from the intestinal tract. *Salmonella* can also be present on pig skin before slaughtering and may subsequently be recovered on the carcass. The presence of *Salmonella* on pig skin, may on its behalf originate from intestinal infection of the pig or from a contaminated environment on the farm, in the transport vehicle or in the slaughterhouse lairage.

The analyses results indicate that processing slaughter pigs that are not infected with *Salmonella* reduces the risk of subsequent carcass surface contamination. Therefore, controlling the *Salmonella* prevalence in pigs during primary production (i.e. from farm to slaughtering) would have a beneficial impact on *Salmonella* contamination of carcasses and pig meat. These controls are also likely to reduce the overall *Salmonella* contamination of slaughterhouse environment, since incoming pigs are the primary source of *Salmonella* ingress to slaughterhouses.

The survey results also underline the role of the slaughterhouse environment in *Salmonella* carcass contamination. Even though a pig infected in the lymph nodes was more likely to yield a contaminated carcass, there were many contaminated carcasses deriving from pigs with lymph nodes tested negative. Some of these may be due to limited testing sensitivity to detect all *Salmonella* positive pigs but others may result from cross-contamination from other carcasses or through contact with contaminated surfaces or equipment within the slaughterhouses. Consequently, good slaughter hygiene is also vital in the prevention of carcass *Salmonella* contamination.

5.1.3. Effect of the slaughterhouse on the risk of carcass contamination

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 considerably between slaughterhouses, even when other factors such as lymph node infection, month of sampling and delay in testing were taken into account in the statistical model. This slaughterhouse effect can be interpreted as the combined results of all the other factors affecting the likelihood of the carcass becoming contaminated with *Salmonella* within the individual slaughterhouse. Such factors are likely to include - among other things – the *Salmonella* contamination of slaughterhouse environment, hygiene during slaughter and subsequent processing, slaughter techniques, the speed of the slaughter line and the cleaning and disinfection procedures used.

Furthermore, the analysis showed that, depending on the slaughterhouse, *Salmonella* infection in pigs arriving on the slaughter line has either a stronger or weaker impact on carcass contamination. In some slaughterhouses carcasses were more likely to become *Salmonella* contaminated than in others, both when processing infected or non-infected slaughter pigs. Apparently certain slaughterhouses were more capable of controlling and preventing *Salmonella* contamination risk in the slaughter process. This implies that while slaughterhouse and the processing steps offer a further opportunity for *Salmonella* risk mitigation in pig, they may also contribute to increase the risk, notably in case of poor hygienic performances.

The survey did not collect information on slaughterhouse characteristics that could have contributed to the contamination of carcasses. However, it may be in the interest of MSs to investigate further these specific slaughterhouse factors in order to improve the control of *Salmonella* and the protection of public health in their country.

5.1.4. Effect of the time of sampling on *Salmonella* results

Throughout the survey an association was found between *Salmonella* contamination of pig carcasses and the time of sampling. Carcasses were less at risk of being contaminated during the first months of the survey, October 2006 to March 2007, compared to the rest of the survey period, from April to September 2007. This effect could be due to the season, but this hypothesis should be studied further and confirmed by additional pluriannual studies conducted in MSs, particularly since seasonal climatic conditions differ between MSs across the EU. The effect of the sampling months on the risk of surface contamination of carcasses was previously described in the literature. Mc Dowell et al. (2007) reported, in Northern Ireland, that the highest odds of carcass contamination occurred in the quarter April to June and the lowest in October to December. A study carried out in five slaughterhouses from three European countries also showed that the risk of carcass contamination was significantly higher in the summer compared to the autumn months (Hald et al., 2003).

In the analyses, there was no evidence that time of sampling during the day was associated with the risk of *Salmonella* infection of lymph nodes or carcass contamination.

5.2. Analysis of serovar and phage type distribution

5.2.1. Spatial distribution of *Salmonella* serovars in lymph nodes

Spatial distribution analysis identified likely clusters of MSs, representing geographical areas where infection with a particular serovar was significantly higher than in the general EU slaughter pig population. The only geographical information available for the analysis was the MS and therefore the smallest geographical unit for inclusion or exclusion in a cluster were individual MSs. This analysis implied that all *Salmonella* isolations occurred at a central point within the MS. This is obviously a gross simplification and the analysis thus investigated the adjacency of MSs in which positive pigs occurred, rather than the true geographical relatedness of positive pigs. Nevertheless, the outcomes were consistent with the visual appraisal of MS-specific prevalence and show that various serovars isolated from slaughter pigs are not evenly distributed across the EU. *S.* Typhimurium and *S.* Derby are clustered in western MSs, whereas *S.* Enteritidis is clustered in eastern MSs. *S.* Infantis appeared to cluster in north-eastern MSs, while *S.* Rissen clustered in the Iberian peninsula.

The clustering of *Salmonella* serovars in specific geographic areas may mirror common sources or reservoirs of infection such as endemic wildlife species, specific raw feed ingredients, or indeed, infected breeding herds of pigs. Geographic clustering is also consistent with the potential for the clonal spreading of a particular *Salmonella* serovar among holdings following the introduction to a region, e.g. through the movement of infected animals, or through feed or animal transport vehicles. Clustering may also reflect a selection pressure for a specific serovar in a region.

The present survey design did not collect data on various relevant factors that could explain more in-depth the identified clusters, such as feed ingredients, production managerial procedures, farm characteristics and pig movements, as well as the *Salmonella* status of the suppliers of piglets or breeding animals; nor holding bio-security such as access to wild or other domestic livestock. However, one hypothesis may be that the differences in pig farming structure could partly explain the observed differences, with larger and more industrialised productions in western MSs and more extensive and mixed productions in eastern MSs. MSs where particular serovars are prevalent should attempt to identify specific risk and/or protective factors enabling appropriate control measures in their country.

5.2.2. Comparison of serovar and phage type distribution in slaughter pigs, feed and human salmonellosis cases

There were substantial variations among MSs in *Salmonella* serovars detected from pigs in this survey, as stated in Part A report. This serovar distribution was analysed and compared to those in human salmonellosis cases, in other food production animal species, and in feed. The analyses of *S.* Enteritidis and *S.* Typhimurium phage types was carried out as well, but it proved less useful because the phage type data was only available from few MSs.

In this survey, *S.* Typhimurium dominated the serovars isolated from pigs at EU level, and this serovar is also clearly the second most often reported serovar from human cases, following *S.* Enteritidis. According to analyses, in most MSs, where *S.* Typhimurium presented an important proportion of the serovars found in slaughter pigs, the serovar was also the dominant cause of human non-Enteritidis *Salmonella* infections. This supports the notion that pig meat may contribute to the human *S.* Typhimurium infection in EU.

There also appeared to be some agreement between the human and pig proportion of *S.* Derby and *S.* Infantis serovars at MS level, even though some discrepancies were observed. This can be expected as these human cases of these serovars are likely to represent infections from many different sources and food chains. Generally, many serovars isolated from slaughter pigs in this survey (such as *S.* Enteritidis, *S.* Infantis and *S.* Livingstone) have also been isolated from broilers and laying hens, while *S.* Derby, *S.* Bredeney and *S.* London were shared with turkeys.

Whereas there is general acceptance of a substantial contribution of pig meat to *Salmonella* cases in humans, particularly regarding *S.* Typhimurium infection (Berends et al., 1998; Hald et al., 2004; EFSA, 2006), the true attribution of risk arising from pig meat remains unknown at EU level. While it is known that also other food producing animal species (e.g. poultry and cattle) and the food thereof are sources of *S.* Typhimurium and other *Salmonella* serovar infections in humans, a more in-depth source attribution analysis is needed to examine the relative contribution of the animal species. The on-going Quantitative Risk Assessment on *Salmonella* in pigs that is carried out by EFSA's Scientific Panel on Biological Hazard may contribute importantly in this aspect. In addition, a thorough phage typing and a molecular typing of all *Salmonella* isolates from humans, food and food producing animals would facilitate a better understanding of attribution of risk to specific food chains.

When considering the sources of *Salmonella* in pigs, it was interesting to note, that those MSs, which had a higher prevalence of *S.* Enteritidis in slaughter pigs in the survey, had reported also a relatively high *S.* Enteritidis prevalence in laying hen holdings and/or in broilers flocks in the

previous baseline surveys. This might be indicative of either a common source of this serovar or its circulation between these food animal sectors. Also, a number of serovars detected in the slaughter pigs in the survey have been also isolated from feed. Feed is a plausible and well-recognised source of introduction of *Salmonella* into pig herds, particularly in the case of new serotypes that may be able to establish themselves in pig production

6. Conclusions

- In the survey, a positive association between the frequency of slaughter pigs infected with *Salmonella* in their lymph nodes and the frequency of *Salmonella* surface contamination of pig carcasses was observed. A *Salmonella* infected pig was twice more likely to yield a *Salmonella* contaminated carcass. However, contaminated carcasses could also derive from uninfected pigs.
- The risk of pig carcasses becoming contaminated with *Salmonella* also varied significantly between slaughterhouses even when other associated factors, such as the frequency of infected slaughter pigs, were accounted for. In some slaughterhouses the risks of producing a contaminated carcass from a *Salmonella* infected pig or from a non-infected pig were higher. This indicates that certain slaughterhouses were more capable of controlling and preventing *Salmonella* contamination than others.
- At EU level, pig carcasses were most likely to become contaminated with *Salmonella* in the second half of the survey period, from April to September 2007. However, this possible seasonal effect should be verified in further studies in individual MSs.
- There was substantial variation between MSs in the factors found associated with *Salmonella* infection of slaughter pigs and carcass contamination. Also the level of importance of these factors varied, and while sometimes the same factor could be protective in some MSs, it could increase the risk in others.
- A number of factors such as those related to rearing and processing were not investigated in the survey. Therefore, it was not possible to estimate the association of these factors with *Salmonella* infection of pigs or contamination of carcasses and their potential confounding role on the effect of factors on which data were available. However, results of this analysis are useful starting points for more specifically aimed studies in the EU and in individual MSs.
- The manner in which sampling and testing procedures were applied in the survey affected the likelihood of detecting *Salmonella* from the lymph node samples and the carcass surface samples. The likelihood of detection was highest when there was some delay between sampling and the start of laboratory testing. In addition, the probability of finding *Salmonella* from the lymph nodes increased with the weight of the samples.
- The analyses of the *Salmonella* serovar distribution revealed some agreement between the most frequently reported serovars in human salmonellosis and those isolated in slaughter pigs. This supports the notion that pigs and pig meat contribute to *Salmonella* infection in humans, even though it is acknowledged that other food animal species and food thereof also play a role as a source of these infections in humans.
- Analysis of serovar distributions indicated the clustering of specific serovars in pig production chains of geographic regions within the EU. This clustering may indicate common sources of these serovars among the MSs in question.

7. Recommendations

- MSs are invited to consider the factors found to be significantly associated with *Salmonella* infection in slaughter pigs and contamination on carcasses at EU level in this survey, when designing national *Salmonella* control programmes for slaughter pigs. The *Salmonella* infection status of the pig (reflected by the lymph node infection) and the slaughterhouse process were both shown to have an impact on the risk of carcass contamination. An integrated control programme that addresses both the primary production and the slaughter process may prove to be a feasible and cost-effective option.

- MSs are specifically encouraged to guarantee *Salmonella* controls in primary production as in the slaughterhouses in order to prevent subsequent contamination of the carcass surface and to improve protection at public health.

- The EU pig meat industry is invited on its part to pay increased attention to slaughter hygiene and other factors in slaughterhouses that may affect *Salmonella* contamination of pig carcasses.

- It is recommended that MSs carry out further national studies to identify more closely the factors that put slaughter pigs and carcasses at risk of becoming infected or contaminated with *Salmonella* in their country, taking into account their national *Salmonella* prevalence, serovar distribution and the characteristics of their slaughterhouses.

- The harmonisation of sampling and testing procedures should be considered of importance by MSs when designing national *Salmonella* control programmes, as well as by EU legislation when defining the target for the reduction of *Salmonella* prevalence in slaughter pigs.

- Since the probability of isolating *Salmonella* from a lymph node sample or a carcass swab varied according to the delay between sample collection and laboratory testing, MSs are invited to carry out studies on the survival rates of *Salmonella* in different relevant matrices.

Task Force on Zoonoses Data Collection members

Andrea Ammon, Alenka Babusek, Marta Bedriova, Karin Camilleri, Georgi Chobanov, Mariann Chriel, Inrid Dan, Jürg Danuser, Kris De Smet, Marie Edan, Matthias Hartung, Birgitte Helwigh, Merete Hofshagen, Sarolta Idei, Patrícia Inácio, Elina Lahti, Lesley Larkin, Peter Much, Edit Nagy, Lisa O'Connor, Rob A.A. van Oosterom, Jacek Osek, José Luis Paramio Lucas, Antonio Petrini, Christodoulos Pipis, Saara Raulo, Tatjana Ribakova, Antonia Ricci, Petr Šatrán, Snieguole Sceponaviciene, Joseph Schon, Jelena Sõgel, Ana María Troncoso González, Kilian Unger, François Veillet, Luc Vanholme, and Dimitris Vourvidis.

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Abbreviations

CI	Confidence Interval
CRL	Community Reference Laboratory
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EU	European Union
MS(s)	Member State(s)
NRL	National Reference Laboratory
OR	Odds Ratio
RDNC	'Reacts but Does Not Conform'
RR	Relative Risk
TESSy	European Surveillance System
VIF	Variance Inflation Factor

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