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)

Adopted on 14 November 2008

[1] For citation purposes: Report of the Task Force on Zoonoses Data Collection on the Analysis of the baseline survey on the prevalence of *Salmonella* in slaughter pigs, Part B, *The EFSA Journal* (2008) 206, 1-111

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.

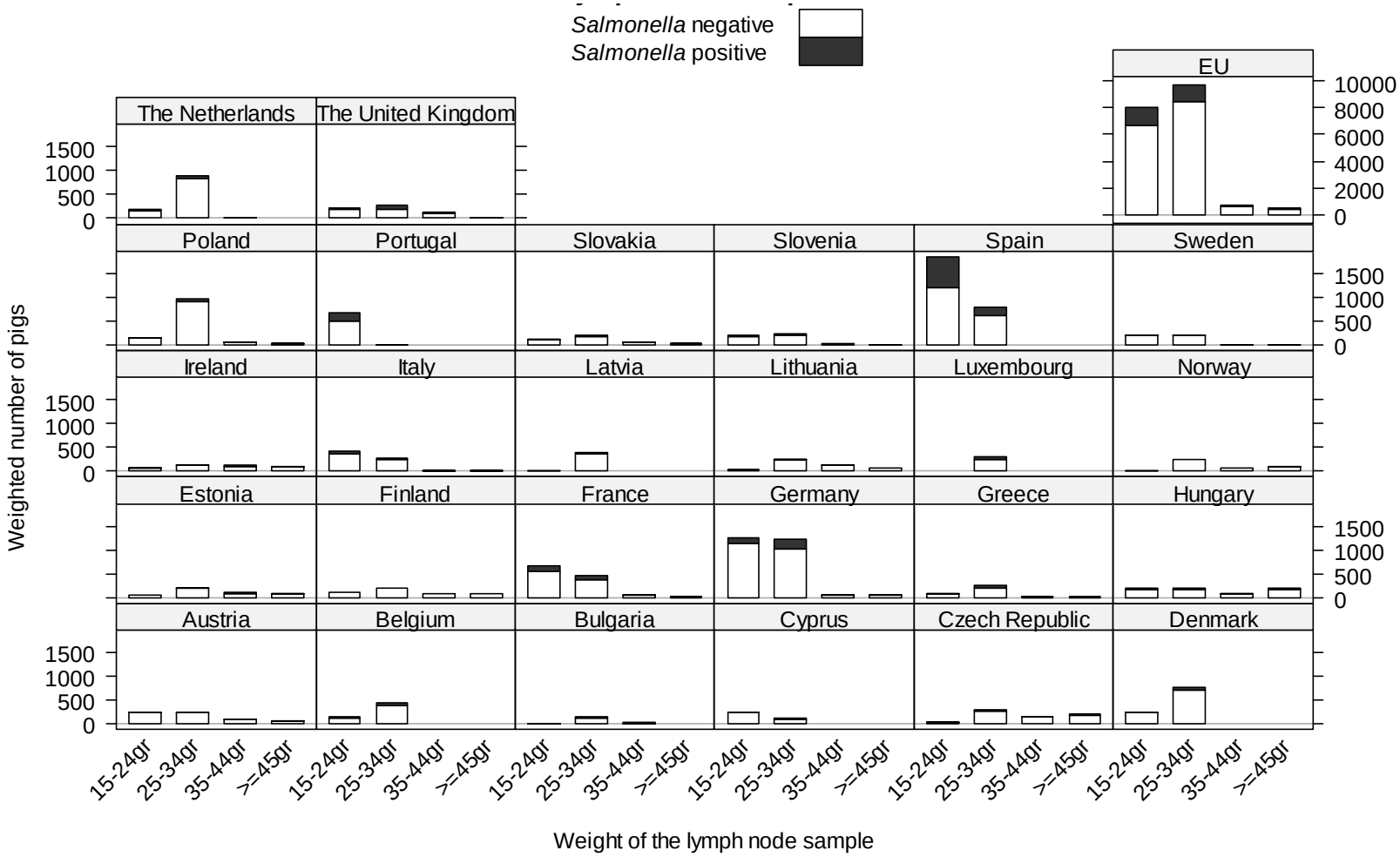
4.1.1. Descriptive analysis of factors potentially associated with *Salmonella* infection

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

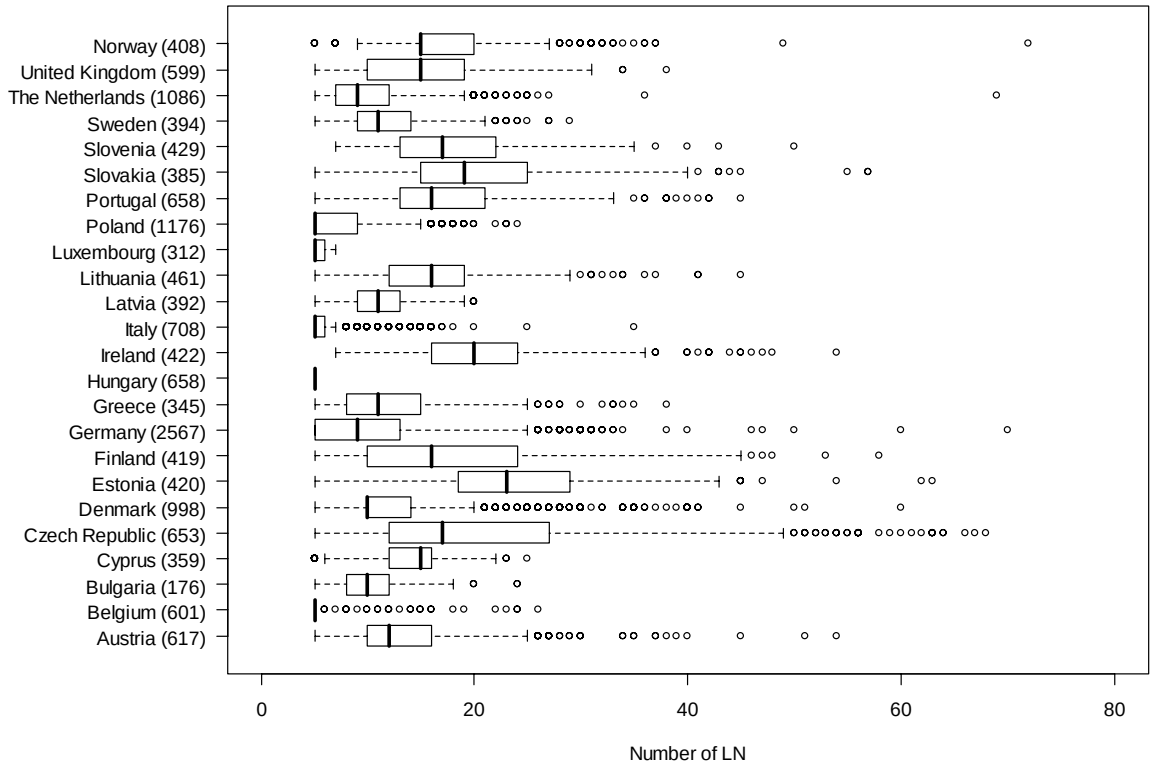


- *Number of lymph nodes in the sample*

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

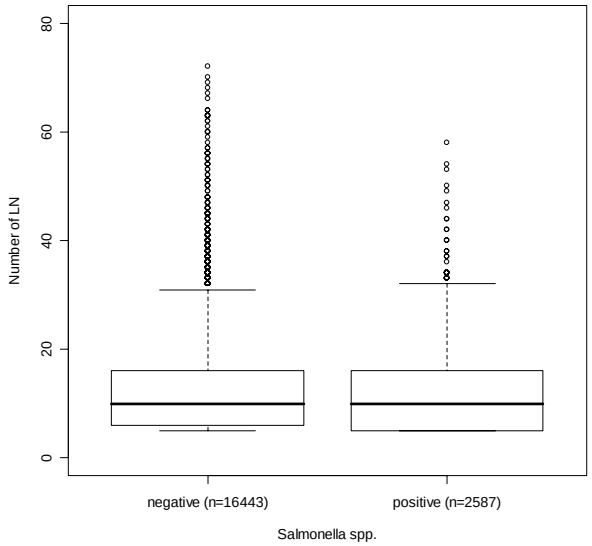
The number of sampled slaughter pigs per MS is indicated between brackets.



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).

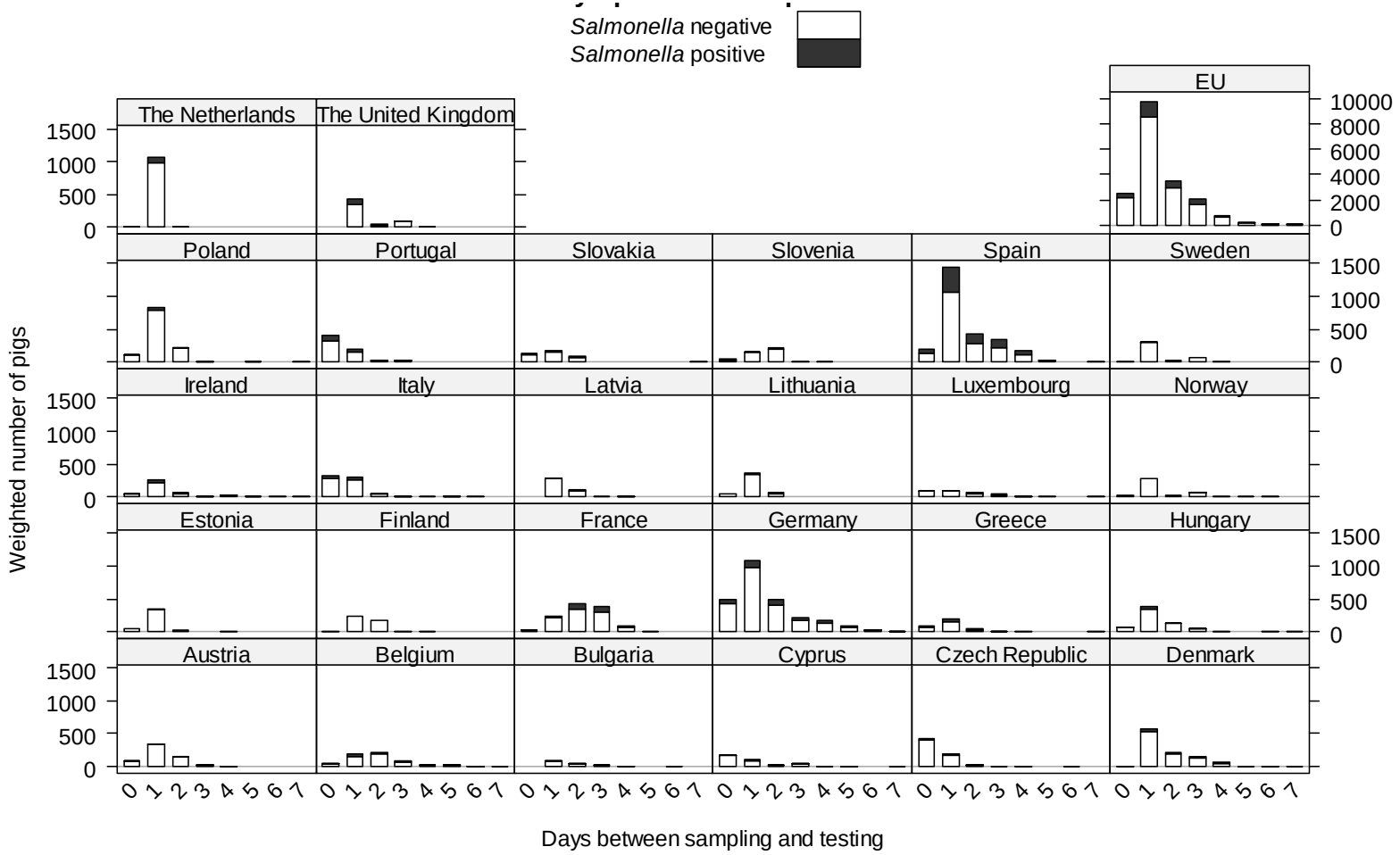
Figure 3. Box plot of the number of lymph nodes per sample by *Salmonella* status of sample



- *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.

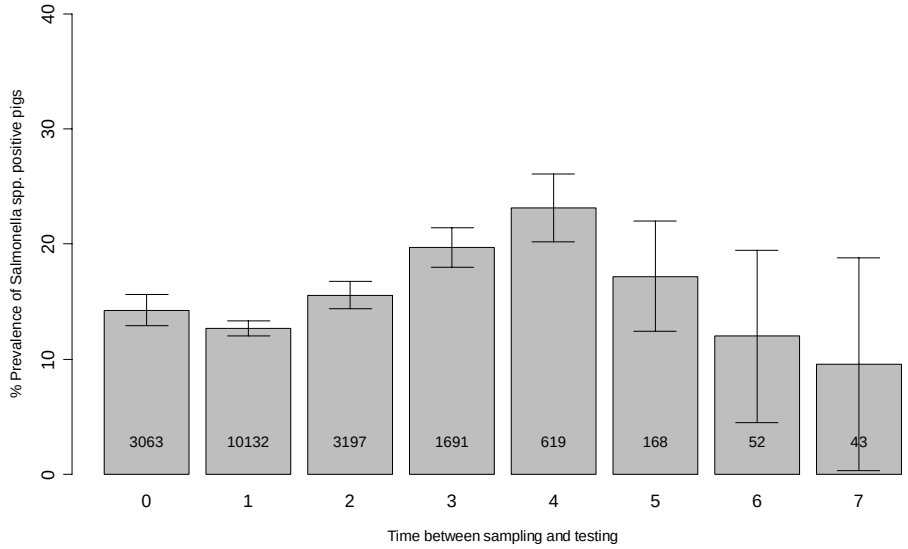
Figure 4. Frequency distribution of the weighted number of days between sampling and testing of lymph node samples, by country and for the EU, and by *Salmonella* status



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.

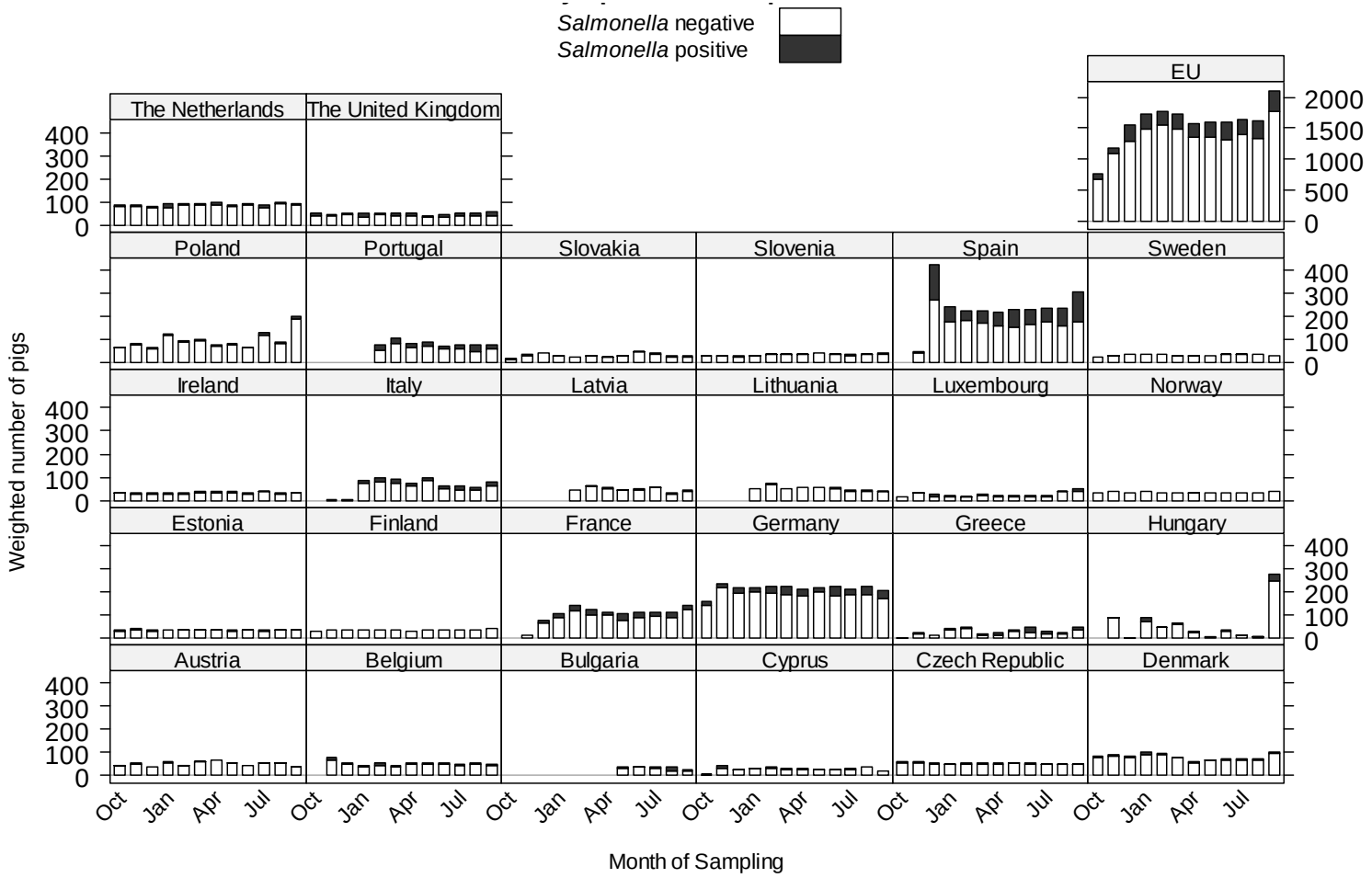
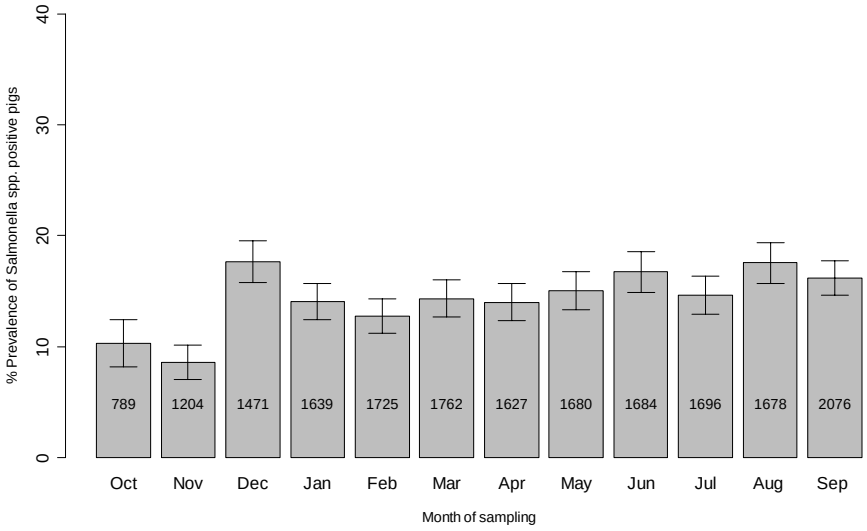


Figure 7. Weighted *Salmonella* prevalence by the month of sampling, with 95% confidence intervals, in the EU

The number of sampled pigs is indicated inside each bar.

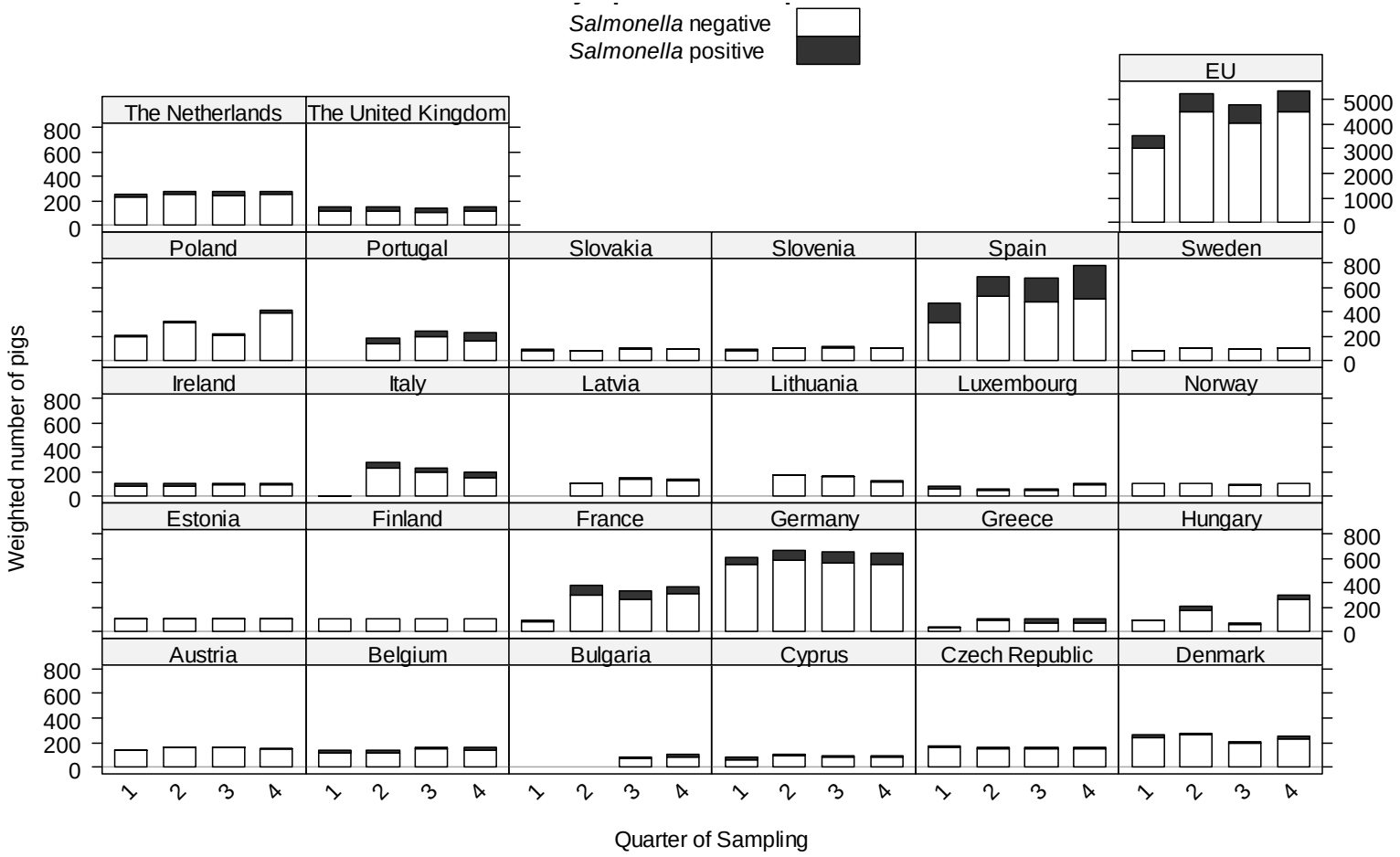


- *Sampling quarter*

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

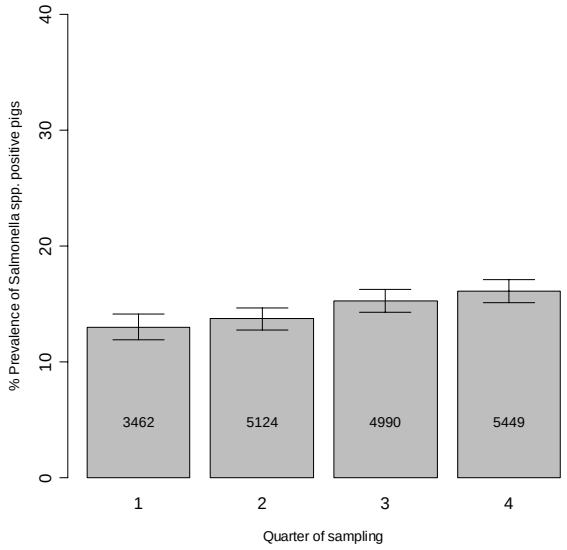
Quarters are ordered from October-December 2006 (1) to July-September 2007 (4).



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.