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SCIENTIFIC REPORT OF EFSA

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

Part B: Analysis of factors associated with *Campylobacter* colonisation of broiler batches and with *Campylobacter* contamination of broiler carcasses; and investigation of the culture method diagnostic characteristics used to analyse broiler carcass samples.

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ABSTRACT

A European Union-wide baseline survey on *Campylobacter* in broiler batches and on broiler carcasses was carried out in 2008. From each of the 10,132 randomly selected batches, pooled caecal contents samples of slaughtered broilers were taken in 561 slaughterhouses and examined for the presence of *Campylobacter*. From the same batches one carcass was collected after chilling, from which neck skin together with breast skin was examined for the presence of *Campylobacter* and the *Campylobacter* count was determined. Multivariable regression analysis showed that a *Campylobacter*-colonised broiler batch was about 30 times more likely to have the sampled carcass contaminated with *Campylobacter*, compared to a non-colonised batch. Also, a higher *Campylobacter* count on carcasses was strongly associated with *Campylobacter* colonisation of the batch. Contaminated carcasses could also derive from non-colonised broiler batches. Both the risks for *Campylobacter*-contaminated carcasses and for *Campylobacter* colonisation of batches increased with the age of the slaughtered broilers as well as during certain months of the year - with the period July-September being the quarter at most risk. Processing later during the day increased the risk of *Campylobacter* contamination of carcasses. Batches originating from previously thinned flocks were more at risk of being colonised with *Campylobacter*. The risks for contamination of carcasses with *Campylobacter*, for higher *Campylobacter* counts on carcasses and for colonisation of batches with *Campylobacter* all varied significantly between countries and between slaughterhouses within countries, even when other associated factors were accounted for. Investigation of the culture method results used to estimate the prevalence of *Campylobacter*-contaminated broiler carcasses showed that the diagnostic sensitivity of the detection test may have varied between Member States.

KEY WORDS

Campylobacter, broiler batches, broiler carcasses, chicken, baseline survey, risk factors, EU.

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SUMMARY

A European Union-wide baseline survey on *Campylobacter* in broiler batches and on *Campylobacter* and *Salmonella* on broiler carcasses was carried out in 2008. A total of 10,132 broiler batches were sampled from 561 slaughterhouses in 26 European Union Member States, plus Norway and Switzerland. From each randomly selected batch, the caecal contents of 10 slaughtered broilers were collected, pooled and examined for the presence of *Campylobacter*. From the same batch, one carcass was collected after chilling from which the neck skin together with the breast skin was examined for the presence of *Campylobacter* or *Salmonella*, and to determine the *Campylobacter* count. *Campylobacter* was detected in pooled caecal contents of broilers and on broiler carcasses in all participating countries. The results of the analysis of *Campylobacter* and *Salmonella* prevalence have already been published by the European Food Safety Authority on 17 March 2010 in the Part A report. The present Part B report provides the results of the associations of eight batch- or slaughterhouse level factors and *Campylobacter* colonisation of batches and/or contamination of carcasses. The investigated prevalence was the observed prevalence, meaning that the prevalence estimates did not account for imperfect test characteristics. In addition, further analyses of the identified *Campylobacter* species distribution across the European Union, and the results of the diagnostic sensitivity investigation of the detection method used to estimate the prevalence of *Campylobacter*-contaminated broiler carcasses, are included in the current report. A Part B report on *Salmonella* will be published at a later stage.

Multivariable regression analysis showed a strong positive association at European Union level between the likelihood of *Campylobacter* contamination of broiler carcasses with the presence of a *Campylobacter*-colonised batch. A *Campylobacter*-colonised broiler batch was about 30 times more likely to have the sampled carcass contaminated with *Campylobacter*, compared to a non-colonised batch. Also, a higher *Campylobacter* count on carcasses was strongly associated with *Campylobacter* colonisation of the batch. These findings indicated an important effect of *Campylobacter* colonisation of broiler flocks on both the frequency of occurrence as well as on the contamination levels of *Campylobacter* on carcasses. Contaminated carcasses could also derive from non-colonised broiler batches, suggesting a potential for cross-contamination in the slaughterhouse environment.

The analyses further showed that the risk for *Campylobacter*-contaminated carcasses increased with the age of the slaughtered broilers, with processing later during the day and during certain months of the year, with the period July-September being the quarter at most risk. The analyses also showed that the time (in hours) between sampling and testing increased the risk of detecting *Campylobacter* from the carcass samples. For country-groups having a prevalence lower or higher than the EU median[4], there was variation in the factors found associated with *Campylobacter* contamination of broiler carcasses and also in the level of importance of these factors. More factors were significant for the group of countries having a higher prevalence.

The risks for contamination of carcasses with *Campylobacter* and for higher *Campylobacter* counts on carcasses varied significantly between countries and between slaughterhouses within countries, even when other associated factors, such as the prevalence *Campylobacter*-colonised batches, were accounted for. These findings indicate that certain slaughterhouses are more capable than others in preventing *Campylobacter* contamination and in controlling the contamination and/or the *Campylobacter* counts on the carcasses. This implies that slaughterhouse processing offers an opportunity for *Campylobacter* risk mitigation.

Factors that were included in the analysis, but which were not significantly associated with *Campylobacter* contamination of carcasses were flock production type, thinning of flocks, capacity of slaughterhouse, type of chilling of the carcass, and *Salmonella*-contamination results on the broiler

[4] Two non-MSs, Norway and Switzerland, were included to calculate the EU median prevalence.

carcass. Moreover, the analyses of the *Campylobacter* contamination carcasses showed that, in the case of *Campylobacter*-non-colonised broiler batches, 18% of the unexplained variance (in *Campylobacter*-contamination results) was attributable to slaughterhouse-specific factors for which no data were gathered during the survey. For *Campylobacter*-colonised broiler batches, this proportion was higher (50%). In the case of the analyses of the counts on contaminated carcasses, the only potential risk factor was the *Campylobacter* colonisation status of the batch. Factors that were included in the analysis but that were not significantly associated with higher counts of *Campylobacter* on carcasses, were flock production type, thinning of flocks, age of broilers, quarter of sampling, time (hour) of processing during the day, time (in hours) between sampling and testing, capacity of slaughterhouse, type of chilling of the carcass, and *Salmonella*-contamination results on the broiler carcass. For some of the factors the power of the analyses was low due to too few samples in some specific categories.

The analyses of the batch level prevalence survey showed that slaughter batches of previously thinned flocks were at a significantly higher risk of colonisation by *Campylobacter*. Age of broilers also emerged as a risk factor, and the risk of *Campylobacter* colonisation of batches increased with the age of the slaughtered broilers and during certain months of the year, with the period July-September being the quarter at most risk. The analyses also showed that the time (in hours) between sampling and testing increased the risk of detecting *Campylobacter* from the caecal contents samples. There was no variation in the factors found associated with *Campylobacter* colonisation of broiler batches for country-groups having a lower or higher prevalence than the EU median. The risk of *Campylobacter* colonisation of batches also varied significantly between countries and between slaughterhouses within countries, even when other associated factors were adjusted for.

Factors that were included in the analysis, but which were not significantly associated with *Campylobacter* colonisation of batches were flock production type and the time (hour) of sampling during the day. For some of these factors the power of the analyses was low due to too few samples represent in some specific categories. Moreover, the analyses showed that 25% of unexplained variance (in *Campylobacter*-colonisation results) was attributable to slaughterhouse-specific factors for which no data were gathered during the survey.

Investigation of the culture methods results used to estimate the prevalence of *Campylobacter*-contaminated broiler carcasses showed that the diagnostic sensitivity of the detection test may have varied between Member States. Thus the true prevalence of *Campylobacter*-contaminated carcasses might be underestimated for some Member States. Consequently, caution is needed when interpreting the results of the European Union level analyses of factors associated with the prevalence of *Campylobacter*-contaminated broiler carcasses and of *Campylobacter*-colonised broiler batches, because the analyses did not correct for test (misclassification) bias.

It is recommended that Member States consider the factors found to be associated with *Campylobacter*-contaminated broiler carcasses and/or with *Campylobacter*-colonised broiler batches at EU level in this survey, when they are designing national *Campylobacter* control programmes for broiler meat or broiler flocks. An integrated control programme that addresses both the primary production and the slaughter process would seem to be important in strategies to prevent or reduce subsequent contamination of the broiler carcass and to improve protection of public health. Further national studies to identify more closely, at batch- and slaughterhouse level, the factors that put broiler batches and carcasses at risk of becoming respectively colonised or contaminated with *Campylobacter* in a country are recommended. The standardisation of the time between sampling and testing, as well as of the quality control of laboratory testing methods, should be considered of importance by Member States when designing national *Campylobacter* control programmes. In particular, it is recommended that Member States investigate further the sensitivity of the *Campylobacter* detection method.

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BACKGROUND

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

Upon a request from the European Commission, the European Food Safety Authority (EFSA) adopted a "Report of the Task Force on Zoonoses Data Collection on proposed technical specifications for a co-ordinated monitoring programme for *Salmonella* and *Campylobacter* in broiler meat in the EU (EFSA, 2007)".

Previously, a Commission Task Force of scientific experts in collaboration with EFSA prepared technical specifications for a baseline study on a harmonised monitoring of *Campylobacter* in broiler flocks.

Based on the EFSA proposal and the Commission technical specifications, the Commission adopted Decision 2007/516/EC[6] of 19 July 2007 concerning a financial contribution from the Community towards a survey on the prevalence and antimicrobial resistance of *Campylobacter* spp. in broiler flocks and on the prevalence of *Campylobacter* spp. and *Salmonella* spp. on broiler carcasses to be carried out in Member States (MSs). This large survey consisting of two sub-surveys started on 1 January 2008 for a period of 12 months.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

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

EFSA is asked to analyse the results of the baseline survey on *Campylobacter* in broiler flocks and on *Campylobacter* and *Salmonella* on broiler carcasses, in particular:

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

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

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

ANALYSIS

1. Introduction

This report (part B) describes the results of a baseline survey carried out in the EU to estimate the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses at slaughterhouse level. This survey was the sixth in a series of baseline surveys carried out within the EU and it was the first baseline survey directly investigating foodstuffs. The objective of the survey has been to obtain comparable data for all MSs through harmonised sampling schemes. According to Article 5 of Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents[7], such surveys may be established, especially when specific needs are identified, to assess risks and to establish baseline values related to zoonoses and zoonotic agents at the level of MSs. Results of such a survey will inform of the need of Community-wide intervention.

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

Two part B reports are produced regarding this baseline survey. The present report part B provides the EU level analyses of factors associated with *Campylobacter*-colonised broiler batches and with *Campylobacter*-contaminated broiler carcasses, further analyses of the identified *Campylobacter* species distribution across the EU, as well as the results of the investigation of the diagnostic characteristics of the detection and enumeration methods applied to estimate the prevalence of *Campylobacter*-contaminated broiler carcasses. A second, separate part B report will describe the analyses of factors associated with *Salmonella*-contaminated broiler carcasses, as well as more in-depth analyses of *Salmonella* serovar distributions, and will be published at a later date. The results of the antimicrobial susceptibility of the *Campylobacter* and *Salmonella* isolates from the survey will be evaluated, in accordance with Article 9 of Directive 2003/99/EC, in the annual report on trends and sources of zoonoses, zoonotic agents and antimicrobial resistance in the European Union.

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

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

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

7 Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EC and repealing Council Directive 92/117/EC. OJ L 325, 12.12.2003 p. 31.

2. Definitions

In the scope of this baseline survey and report the following definitions were identified:

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

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

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

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

***Campylobacter*-colonised broiler batch**: a broiler batch from which *Campylobacter* spp. was isolated from the intestines of at least one broiler. This isolation is based on detection of *Campylobacter* spp. from a pooled sample composed of the caecal contents from 10 broilers belonging to the batch using the prescribed culture method.

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

(Diagnostic) sensitivity: means the conditional probability that a *Campylobacter*-contaminated carcass will be positive for the particular survey test.

(Diagnostic) specificity: means the conditional probability that a *Campylobacter*-non-contaminated carcass will be negative for the particular survey test.

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

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

3. Objectives

The primary aim of the survey was to estimate the prevalence of *Campylobacter*-colonised broiler batches and of *Campylobacter*- and *Salmonella*-contaminated broiler carcasses, at EU level and for each MS. The previously published Part A report described the results of the analyses of the prevalence of *Campylobacter*-colonised broiler batches; of *Campylobacter*-contaminated broiler carcasses and of *Salmonella*-contaminated broiler carcasses; the analyses of the *Campylobacter* enumeration results on broiler carcasses; the analyses of the most frequently identified *Campylobacter* species in broiler batches; and *Campylobacter* species and *Salmonella* serovars on broiler carcasses.

The specific objectives related to this part B report were:

- to investigate the effects of factors, which may be associated with *Campylobacter*-colonised broiler batches, at EU level;
- to investigate the effects of factors, which may be associated with *Campylobacter*-contaminated broiler carcasses, at EU level;
- to investigate the association between the prevalence of *Campylobacter*-colonised broiler batches and the prevalence of *Campylobacter*-contaminated broiler carcasses;

- to investigate the slaughterhouse-specific effects on *Campylobacter*-colonised broiler batches and on *Campylobacter*-contaminated broiler carcasses;
- to investigate the effects of factors, which may be associated with counts of *Campylobacter* on contaminated broiler carcasses, at EU level;
- to investigate the identified *Campylobacter* species distribution across the EU;
- to investigate the impact of the detection and enumeration diagnostic test characteristics on the estimated prevalence of *Campylobacter*-contaminated broiler carcasses.

4. Materials and methods

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

4.1. Survey design

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

From each randomly selected batch the intact caecal contents of 10 slaughtered broilers were collected for the detection of *Campylobacter*. In addition, from the same batch, one whole carcass was collected immediately after chilling, but before freezing, cutting or packaging, for the detection and enumeration (determination of counts) of *Campylobacter* and for the detection of *Salmonella*. At the laboratory, the caecal contents from the intact caeca from the 10 slaughtered broilers were aseptically removed and pooled to form one composite sample. In the case of the carcass, the neck skin was removed, if present, together with the skin from one side of the carcass (breast skin) avoiding any fat, to make a test portion. Detection and enumeration of *Campylobacter* were performed using the same initial test portion from each sampled carcass.

Isolation and confirmation of *Campylobacter* organisms in caecal contents and on the broiler carcass samples were undertaken as described in ISO 10272-1:2006(E) 'Microbiology of food and animal feeding stuffs — Horizontal method for detection and enumeration of *Campylobacter* spp. Part 1: Detection method' (ISO, 2006a). At least one *Campylobacter* isolate per batch was speciated using phenotypic methods as described in ISO 10272-1:2006(E) or published molecular methods such as Polymerase Chain Reaction (PCR) techniques. The quantitative analysis of *Campylobacter* in the broiler carcass samples was carried out according to ISO/TS 10272-2:2006 'Microbiology of food and animal feeding stuffs — Horizontal method for detection and enumeration of *Campylobacter* spp. Part 2: Colony-count technique' (ISO, 2006b).

The detection of *Salmonella* in the broiler carcass samples was carried out according to ISO 6579-2002(E). 'Microbiology of food and animal feeding stuffs — Horizontal method for the detection of

[8] In Portugal, Malta and Switzerland no sampling was performed for three or more months (only for pooled caecal contents samples).

Salmonella spp.' At least one isolate from each positive sample was typed by the National Reference Laboratories (NRLs) for *Salmonella*, using the Kaufmann-White scheme.

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

4.2. Data description

A detailed description of the validation and cleaning of the dataset carried out was provided in the Part A report. The final cleaned dataset contained data from 9,916 broiler batches sampled in 559 slaughterhouses for the survey on *Campylobacter* in broiler batches, and from 10,017 broiler carcasses sampled from 560 slaughterhouses for the survey on *Campylobacter* on broiler carcasses sampled from 561 slaughterhouses in 26 MSs, and in two non-MSs (Norway and Switzerland). The number of samples reported for each sub-survey differed slightly for five MSs. Greece did not carry out the survey.

4.3. Analysis of factors associated with *Campylobacter*-positivity

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

4.3.1. Definition of the outcome variables

The following outcome variables were considered:

- *Campylobacter* spp. colonisation of broiler batches, based on the detection method, as a binary outcome variable (positive/negative);
- *Campylobacter* spp. contamination of broiler carcasses based on the combined detection and enumeration methods (parallel testing): positive in either the detection or enumeration method, as a binary outcome variable (positive/negative); and
- *Campylobacter* spp. counts on contaminated broiler carcasses.

In the Part A report, prevalence of *Campylobacter* spp. (*Campylobacter*), *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*) were presented. In this report the only considered outcome variable was *Campylobacter* spp. This was because there is limited knowledge on any differences between the public health importance of the findings of *C. jejuni* or *C. coli*, whether in batches or on broiler carcasses. Secondly, as reported in the Part A report, the speciation results were not as robust as anticipated, because in general in the survey only one isolate per sample was speciated and this is likely to fail in the indication of samples containing both *C. jejuni* and *C. coli*. Furthermore, the enrichment of samples in the detection method might have favoured the outgrowth of certain *Campylobacter* species and different selective media and incubation periods were used by the laboratories. Finally, isolates from the enumeration method were only to be speciated, on a mandatory basis, when *Campylobacter* was not detected from the same sample by detection method.

However, at batch level, factors associated with *C. coli*-colonised broiler batches were also investigated (binary outcome variable: positive/negative). The rationale for this analysis was that broiler flocks having outdoor access and outdoor standing water would be more likely to be exposed to *C. coli* than flocks with no outdoor access. This hypothesis was underpinned by the results of a case-control study cited in EFSA (2010b) that indicated that recreational water was strongly associated with human campylobacteriosis due to *C. coli*. Furthermore, case-control studies of humans suggest differences in some risk factors for *C. coli* and *C. jejuni* (Gillespie et al., 2002; Doorduyn et al., 2010). Also extensively-reared broilers tend to be slaughtered at an older age and with time *C. jejuni* colonisation is replaced with *C. coli* colonisation (El-Shibiny et al., 2005).

4.3.2. Factors investigated

Data on factors potentially associated with the above-mentioned outcomes were collected using a mandatory questionnaire filled out by the competent authorities, or under their supervision, at the time of sampling in the slaughterhouse. The relevant factors are listed in Table 1 and are described in detail in Appendices B, F and I. Table 1 also shows factors that potentially explained the results for the colonisation of broiler batches as well as for the broiler carcass contamination. Some additional (optional) data and variables were collected on a voluntary basis by MSs. However, the effects of these optional factors could not be evaluated due to the scarcity of data reported.

Table 1. **Factors, collected by a questionnaire and potentially associated with *Campylobacter*-colonised batches, *Campylobacter*-contaminated broiler carcasses and/or *Campylobacter* counts on broiler carcasses, from the baseline survey in the EU[*], 2008**

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

* Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated.

(a): In a conventional flock type birds are housed. A free-range flock system is a flock production type where birds have access to the outside. An organic flock system is a production type that is similar to the free-range system and that fulfils the requirements set out for organic production; birds have access to the outside and are registered with a recognised organic standard regulatory organisation.

(b): Recoded into a new variable 'quarter of sampling'.

(c): Factor related to the sensitivity of the testing process.

During the data analyses certain decisions were made regarding (re-)coding and the use of recorded factors. Firstly, it was decided not to investigate the potential effect of the variable 'selective media used for *Campylobacter* detection' because, as reported in the Report part A and as explained above, different selective media and different incubation methods were used among MSs and laboratories. Secondly, the variable 'date of sampling' was recoded into a new variable 'quarter of sampling' as follows: first quarter - January to March; second quarter - April to June; third quarter - July to September; and fourth quarter - October to December 2008. Thirdly, the age of broilers was considered using a scale of 10 days to assess the risk of *Campylobacter* colonisation or contamination by 10 day increments. Finally, in the list of factors some categorical variables were included which have a natural ordering, such as 'time (hour) of sampling', 'hours between sampling and testing' and 'capacity of slaughterhouses'. Some combinations of classes of these categorical variables were characterised by sparse or no observations, resulting in a problematic fit of regression models. Rather than treating them as nominal, categorical variables, they were used as continuous variables, after having assigned been a score, corresponding to the estimated midpoint of each category, to reflect the distance between each category. This approach is illustrated in Table 2 for the factor 'time (hour) of sampling'. The parameter estimates corresponding to the scores were mainly used to assess the direction of the effect, without giving too much emphasis (interpretation) on the size of the effect.

The factor 'time in hours between sampling and testing' was considered as a factor related to the sensitivity of the testing process and not a potential risk factor per se. Therefore, when this factor was retained in the final regression models, the results were not shown although it was used to adjust those final regression models.

Table 2. **Description of scores for the factor 'time (hour) of sampling during the day'**

Time (hour) of sampling	Estimated midpoint	Scores used in the models
<9 am	6:30 am	6.5
9 - <12am	10:30 am	10.5
12am – <3pm	1:30 pm	13.5
≥3pm	5:30 pm	17.5

For the 'time in hours between sampling and testing' six categories were considered, namely: <24 hours; 24-36 hours; 36-48 hours; 48-60 hours; 60-72 hours and 72-80 hours. The scores used for the 'capacity of the slaughterhouse' are shown in Table 3.

Table 3. **Description of scores for the factor 'capacity of slaughterhouse'**

Capacity of the slaughterhouse	Estimated midpoint	Log_{10} of midpoint	Scores used in the models
<100,000	50,000	4.70	4.7
100,000-499,999	300,000	5.48	5.5
500,000-999,999	750,000	5.88	5.9
1,000,000-4,999,999	3,000,000	6.48	6.4
5,000,000-9,999,999	7,500,000	6.88	6.9
≥10,000,000	15,000,000	7.18	7.2

4.3.3. Exploratory bivariable analysis of potentially associated factors

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

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

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

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

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

The association between each factor and the outcomes of interest were tested separately by Chi-square tests, Spearman correlation and Cochran-Mantel-Haenszel Chi-square tests for linear trends. Due to possible confounding[9] these results should be interpreted cautiously and only within the context of an exploratory analysis.

4.3.4. Identification of factors associated with *Campylobacter*-positivity

Multivariable regression analysis was applied to obtain adjusted estimates of the effect of factors associated with the outcome of interest. The inclusion of multiple factors (predictors) in a regression model allows the adjustment for confounding that may result from association among these factors. Multivariable regression analyses were carried out at EU level. However, in order to investigate the consistency of analyses made at EU level, results from countries with prevalence below and above the median EU[10] prevalence were subjected to additional analyses.

4.3.4.1. Analysis of multicollinearity among potentially associated factors

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

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

10 Two non-MSs, Norway and Switzerland, were included to calculate the EU median prevalence.

4.3.4.2. Statistical model

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

4.3.4.2.1. Aspects of clustering and of weighting of results

The clustering of results could result from several factors. Broiler batches/broiler carcasses, which were the epidemiological units of the analysis, sampled at the same slaughterhouse would have been exposed to the same conditions and risk factors, including those on which no information was available in the current survey but that might have been associated with *Campylobacter* colonisation/-contamination. The rearing and pre-harvest processes, including comparable managerial and hygiene practices of farming and transportation of broiler flocks, are likely to be more similar among broiler batches processed in the same slaughterhouse than among broiler batches processed in different slaughterhouses. Similarly, the contamination by *Campylobacter* of broiler carcasses processed in the same slaughterhouse may also correlate because of common processing and hygiene conditions and potential cross-contamination. Therefore, the risk for *Campylobacter*-positive samples collected at the same slaughterhouse (including both broiler batches and broiler carcasses) may be more similar than for samples collected at different slaughterhouses and such observations cannot be considered as independent observations in statistical analysis. Consequently, correlation among outcomes in those batches/carcasses slaughtered at the same slaughterhouse, which induces extra variation (heterogeneity) between slaughterhouses (clusters), was taken into account in the statistical analysis of the effects of risk factors by including, in the regression model, a slaughterhouse-specific effect (random intercept[11] parameter, which is a random variable representing the effect of factors shared by batches/carcasses slaughtered at the same slaughterhouse) for the outcome of interest (*Campylobacter* slaughter batch colonisation or carcass contamination). The assumption underlying this type of model is that each slaughterhouse, and consequently each batch/carcass processed in that slaughterhouse, is characterised by a certain baseline level of risk of colonisation/contamination, regardless of the exposure to factors considered in the survey. It is noteworthy that the interpretation of the regression coefficients (odds ratios - ORs) in this model is conditional on the slaughterhouse-specific effects and that they cannot be interpreted as describing population-averaged effects of factors. This means that the obtained odds ratios are to be interpreted relative to slaughterhouses having comparable risk factors. Possible country confounding effects were also taken into account in the analysis by including the factor 'country' as a fixed effect into the model. Thus, logistic mixed-effects models were fitted with the effect of the slaughterhouse included as a random effect and the effect of the country as a fixed effect. These mixed-effects models enabled investigating differences in the outcome (*Campylobacter* slaughter batch colonisation or carcass contamination) between slaughterhouses, within countries. More detailed explanations about how to take account of correlation among observations in the statistical analysis of the effects of risk factors can be found in EFSA's 'Report of the Task Force on Zoonoses Data Collection on the statistical analysis of temporal and spatial trends of zoonotic agents in animals and food' (EFSA, 2009a).

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

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

broilers within a country was calculated as the ratio of the number of slaughtered broilers during a year in a country and the number of broilers sampled in the same country.

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

4.3.4.2.2. Model building for *Campylobacter* colonisation/-contamination, at EU level

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

For the analysis of the data from broiler carcasses, records for which the corresponding *Campylobacter*-colonisation result and/or the *Salmonella*-contamination results were missing were discarded.

4.3.4.2.2.1 Analysis of slaughterhouse-specific association between the *Campylobacter* contamination on the broiler carcasses and the *Campylobacter* colonisation in the broiler batches

Because risk factors might have different effects in different slaughterhouses a slaughterhouse-specific random slope was included, - in the logistic mixed-effects models, - for those factors the effect of which was expected to vary across slaughterhouses.

One such factor was the *Campylobacter*-colonisation result within the batches. Therefore a slaughterhouse-specific random slope was added to the mixed-effects model for the factor "*Campylobacter*-colonisation result in the batches". This allowed the investigation of the variation, across slaughterhouses, of the association between batch colonisation and carcass contamination. Any data of broiler carcasses, for which the corresponding batch *Campylobacter*-colonisation results were missing, were deleted from the dataset. In addition, the covariance between the slaughterhouse-specific random intercepts and random slopes was investigated to measure the (linear) dependence between these two random variables.

The quantification of the association between the *Campylobacter*-contamination result on the broiler carcasses and the *Campylobacter*-colonisation result in the broiler batches, was undertaken by investigating the OR covered in the final EU level model for carcass *Campylobacter* contamination , with the *Campylobacter*-colonisation result in the broiler batches as an explanatory variable for the carcass outcome.

With the aim of visualising the variability between slaughterhouses with respect to random effects (random intercept and random slope), as estimated by the final model for *Campylobacter* contamination of carcasses, a scatterplot was produced that displayed for every surveyed slaughterhouse two specific estimates:

- the slaughterhouse-specific baseline effect, which is the sum of the country-specific (fixed) effect and the slaughterhouse-specific effect (the random intercept), while keeping the other factors at a fixed level; and

- the slaughterhouse-specific effect of the factor *Campylobacter* colonisation of the broiler batch (the random slope).

Each slaughterhouse was marked with a dot or a triangle according to whether its estimated prevalence of *Campylobacter*-colonised broiler batches was above or below 50%, respectively. A line further represented the values for the two parameters (random intercept and slope) resulting in a prevalence of *Campylobacter*-contaminated broiler carcasses of 50%.

4.3.4.2.3. Analysis of the variance explained by the slaughterhouses

According to the outcome of the random-effect models, the total variability could be split into two parts: one part explained by the factors and a remaining unexplained part. The latter unexplained variance is due to factors for which no data were gathered during the survey. This unexplained variance was further investigated to quantify the proportion attributable to random effects (slaughterhouse-specific effects). Therefore, the intra-slaughterhouse or intra-cluster correlation coefficient (ICC) was estimated and was approximated as the sum of the variance of the random effects and the variance of the standard logistic density (Molenberghs and Verbeke, 2005). This coefficient could be interpreted as the proportion of the variance, which was unexplained by the investigated factors but which could be explained by the random effects. The ICC provided for the proportion of unexplained variance that was approximately attributable to uninvestigated slaughterhouse-specific factors. An ICC ranges between zero and one and corresponded respectively to scenarios of low (closer to zero) or high (closer to one) proportions of unexplained variance that was due to random effects (slaughterhouse-specific effects, between-slaughterhouse variability). In the latter case the *Campylobacter*-colonisation results of batches or *Campylobacter*-contamination results of broiler carcasses within a slaughterhouse were very much associated (alike). Details on the calculations of the ICC in the context of the used random-effects models are presented in Appendix J.

4.3.4.3. Model building for counts of *Campylobacter* on *Campylobacter*-contaminated carcasses at EU level

A detailed description of the *Campylobacter* enumeration results on carcass samples was provided in the Part A report (EFSA, 2010a). As an exception in this baseline survey, Luxembourg did not perform *Campylobacter* enumeration on carcass samples. Overall (for MSs and the two non-MSs), the percentages of broiler carcass samples with enumeration results (cfu/g of neck skin together with breast skin) below 10, between 10-99, between 100-999, between 1,000-10,000 and above 10,000 were 49.1%, 11.6%, 18.6%, 15.2% and 5.5%, respectively. The median count for carcass samples having at least 1 cfu/g, was 580 cfu/g.

Given the count outcome variable, Poisson regression was the model of choice in the analyses. However, the data were not optimal for such models due to overdispersion. This means that the data values were not randomly distributed across all of the above-mentioned categories of counts. Therefore, a negative, binomial, mixed-effects model was fitted, instead.

Furthermore, with the aim of evaluating factors specifically associated with higher *Campylobacter* counts on broiler carcasses, it was decided to use only data on samples from which countable results (at least 1 cfu/g) were obtained. Consequently, from the total dataset[12] of 10,004 carcass samples with enumeration results, 49% of the records were discarded. Moreover, for this analysis the data from broiler carcasses, for which the corresponding *Salmonella*-contamination results were missing, were deleted from the dataset. In the final dataset 5,074 carcass samples remained.

12 Exceptionally in Luxembourg no *Campylobacter* enumeration was executed in broiler carcass samples.

During the analysis model convergence problems were encountered because of the wide range in counts, between 1 and 1,700,000,000. To deal with this statistical issue the highest count values were recoded and set equal to the 99% quantile of the distribution, which was 150,000. Fifty count values (1% of the dataset) were thus recoded. This data transformation assumed that, in addition to overdispersion, there would also be an issue of measurement error: thus, the precise value of those very high counts was not considered as important in itself, but it was just the indication of a very high count.

Regarding model building, an analogous strategy was implemented as for the prevalence models. First, a full (initial) model investigated all the main effects without any interaction terms (additive model). Next a weighted random-effect model was fitted, where the cluster corresponded to the slaughterhouse. One by one the factors which were non- significant were discarded starting with the largest *P*-value based on the Type III test (Wald's test). Only the factors with *P*-values smaller than 0.05 were retained in the final model. The significance of the random effect was tested using the Wald test and a 50:50 mixture of Chi-square distributions with 0 and 1 degrees of freedom (Molenberghs and Verbeke, 2005).

4.4. Descriptive investigation of the *Campylobacter* detection and enumeration methods test results of broiler carcass samples

Diagnostic tests are not completely accurate and due to test characteristics for sensitivity and specificity being lower than 100% some samples are misclassified to give false-negative or false-positive results respectively. This is also the case for all detection methods that have an imperfect diagnostic sensitivity (Gardner, 2004) and thus results from a microbiological survey can only provide an indication of the observed prevalence. An observed or apparent prevalence estimate does not take account of imperfect test sensitivity or specificity.

In order to improve the interpretation of the estimated EU level and MS-specific observed prevalence of *Campylobacter*-contaminated carcasses set out in Report part A, an investigation was carried out on the impact of possible survey method (test) classification bias on the observed country-specific prevalence of *Campylobacter*-contaminated carcasses. This was done by a detailed descriptive analysis of the *Campylobacter* detection and enumeration methods test results of broiler carcass samples, by country.

4.5. Analysis of *Campylobacter* species frequency distribution

The prevalence of *Campylobacter*-colonised batches and of *Campylobacter*-contaminated broiler carcasses was descriptively compared with the notification rates of human campylobacteriosis cases as reported in the Community Summary Report on Zoonoses in 2008 (EFSA, 2010c). Also the identified *Campylobacter* species distribution, in colonised broiler batches and on broiler carcasses, was descriptively analysed across the EU by the production type (conventional, free-range organic and free-range standard) of the flock of origin.

5. Results

5.1. Factors associated with *Campylobacter*-positivity

5.1.1. Analysis of factors associated with *Campylobacter*-colonised broiler batches

Univariate description and bivariate association factors potentially associated with *Campylobacter*-colonised broiler batches are presented in full in Appendix B (Tables 14-25 and Figures 11-19). The most relevant information is also displayed in the following.

5.1.1.1. Descriptive analysis of factors potentially associated with *Campylobacter*-colonised broiler batches

5.1.1.1.1. Previous thinning of the flock

The weighted prevalence of *Campylobacter*-colonised broiler batches was higher for batches from previously thinned flocks than those from non-thinned flocks (Figure 1).

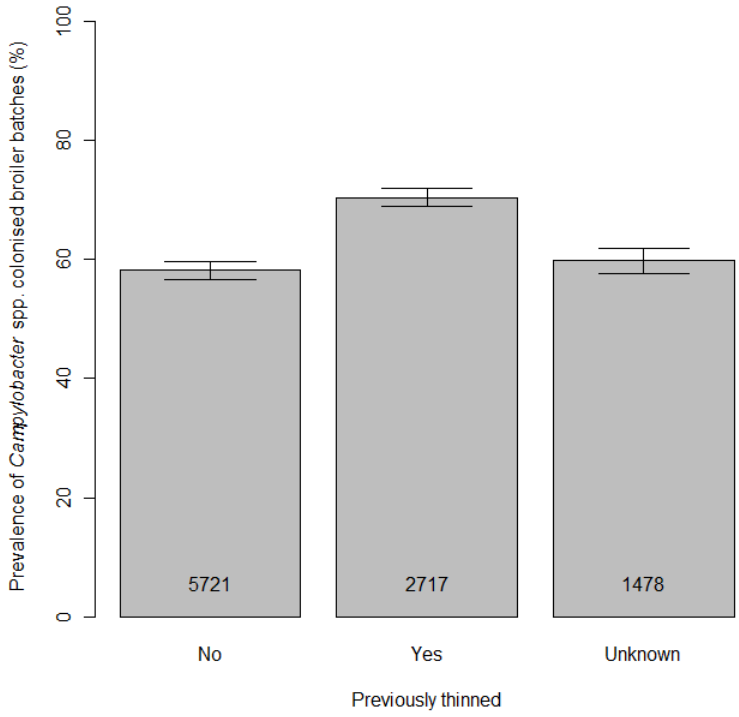

Figure 1. **Prevalence of *Campylobacter*-colonised broiler batches by previous thinning in the flock outcome in the EU*, 2008**

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

5.1.1.1.2. Age of broilers

The median age in days of broilers in *Campylobacter*-colonised broiler batches was greater than the median age of broilers in non-colonised batches (Figure 2). Thus, *Campylobacter*-colonised broiler batches tended to have older birds. There were also a considerable number of outliers for the age especially in the colonised batches group.

5.1.1.1.3. Quarter of sampling

Figure 3 displays a barplot of the weighted prevalence of *Campylobacter*-colonised broiler batches within different quarters of sampling during the year 2008, and their corresponding 95% confidence intervals. Prevalence was highest in the summer period: July, August and September (third quarter), and the lowest in the first three months of the year (first quarter).

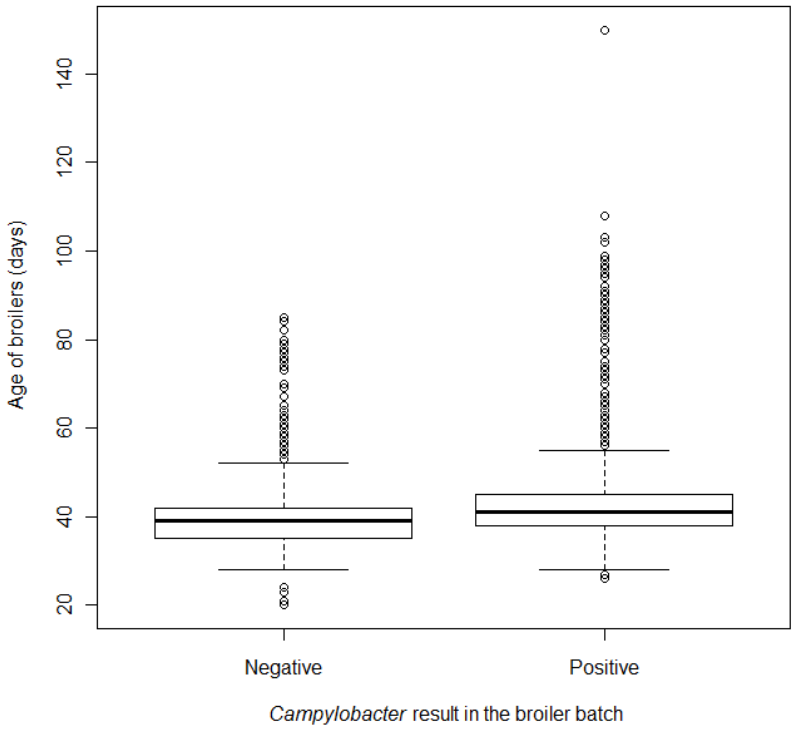

Figure 2. **Boxplot of the age of broilers by *Campylobacter*-colonisation result in the broiler batch, 2008***

* In the boxplots, the bottom section of the box represents the first quartile of the distribution and the top section of the box the third quartile, 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).

5.1.1.2. Analysis of multicollinearity among potentially associated factors related to *Campylobacter*-colonised broiler batches

The VIF values calculated for the multicollinearity analysis among the factors associated with *Campylobacter* colonisation in broiler batches (namely flock production type, previous thinning in the flock, age of broilers, quarter of sampling, time (hour) of sampling and hours between sampling and

testing) are presented in Table 26 (Appendix C). This analysis showed that multicollinearity was not important for the full model since all the VIF values were very small.

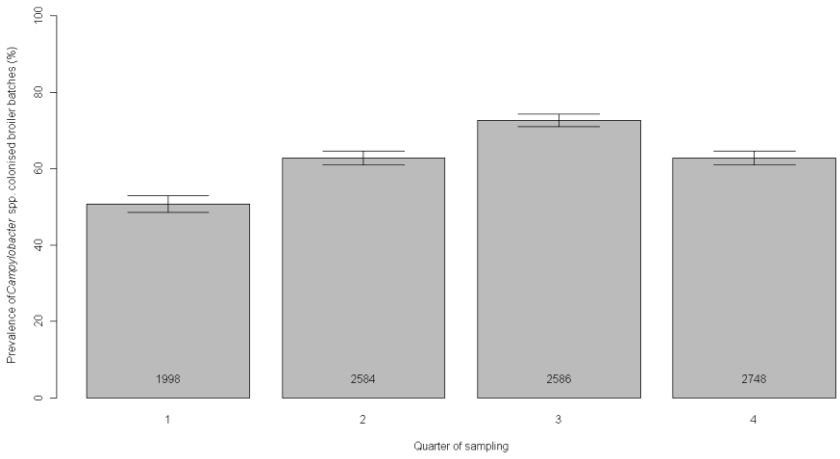

Figure 3. **Prevalence of *Campylobacter*-colonised broiler batches by quarter of sampling in the EU*, 2008**

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

5.1.1.3. Identification of factors potentially associated with *Campylobacter*-colonised broiler batches

A full random-effect model was fitted including all the available factors; country, flock production type, previous thinning in the flock, age of broilers, quarter of sampling, time (hour) of sampling, and time in hours between sampling and testing. The *P*-values for flock production type and time (hour) of sampling during the day were not significant; hence they were discarded from the model based on the backward procedure.

The following potential risk factors for *Campylobacter*-colonised broiler batches were retained in the final logistic mixed-effects model:

- previous thinning in the flock of origin;
- age of broilers (at sampling);
- quarter of sampling.

The OR estimates for factors in the final model at EU level are presented in Table 4.

Table 4. **Final logistic mixed-effect model [(a)] for factors associated with *Campylobacter*-colonised broiler batches, in the EU[*], 2008**

Factor	Level	Odds ratio**	95%CI		*P*-value
Previous thinning in the flock	*Unknown*	1.02	0.75	1.37	<0.0001
Reference category: *No*	*Yes*	1.74	1.36	2.24	
Age of broilers (scale 10 days)		1.98	1.66	2.35	<0.0001
Quarter of sampling	*IV*	2.17	1.66	2.85	<0.0001
Reference category: *I*	*III*	4.07	3.09	5.36	
	II	2.10	1.64	2.69	

(a): Estimates and standard errors were assessed using a mixed-effects model with the effect of slaughterhouses included as a random effect (random intercepts) and with the factor 'country' included as a fixed effect.

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

** All ORs were adjusted for the factors 'country' and 'time in hours between sampling and testing', which were both retained in the final model.

In Table 4, an OR >1 indicates that exposure to the factor increases the risk of *Campylobacter* colonisation, whereas an OR <1 indicates a negative association between the factor and colonisation. An OR equal to 1 indicates no effect of the factor on *Campylobacter* colonisation. Consequently, if the 95% confidence interval (CI) of the OR does not comprise 1, meaning that both the lower and the upper limits are either greater, or less than 1, it can be concluded that the association with a potential factor and *Campylobacter* is statistically significant (*P*-value <0.05). The final model included country-specific effects and the factor 'time (in hours) between sampling and testing' that related to the sensitivity of the testing process (effects not shown). A longer delay increased the risk of detecting *Campylobacter* from the batches. ORs are adjusted for the effects of both factors.

Batches originating from previously thinned flocks were at a higher risk of colonisation compared to non-thinned flocks. Also, batches of older broilers were more at risk of being colonised than batches with younger birds and the risk augmented by a factor of two for every increase of 10 days in the age of the slaughtered broilers. Lastly, broiler batches were more likely to be found *Campylobacter*-colonised in the second, third or fourth quarters of the year compared to the first quarter (January-March). The batches were most likely to be colonised in the third quarter (July-September).

The variance of the random effects (effect of slaughterhouses) in the final regression model was significantly different from zero (*P*-value <0.001, Appendix C, Table 27). This indicated that the baseline risk of *Campylobacter* colonisation of slaughter batches varied between slaughterhouses, even when accounting for other factors i.e. previous thinning in the flock, age of the broilers, quarter of sampling and the factor 'country'. Consequently, within countries, there were slaughterhouses with an overall higher prevalence and slaughterhouses with an overall lower prevalence of *Campylobacter*-colonised batches. The proportion of variance (in *Campylobacter*-colonisation results) that remained unexplained by the investigated factors and that was due to between-slaughterhouse variability was 25% (Table 5). Thus, one fourth of the unexplained variance was attributable to slaughterhouse-specific factors for which no data were gathered during the survey.

An additional complementary analysis following the above modelling approach was made to compare the full model results for *Campylobacter*-colonised broiler batches between two groups of countries having higher and lower batch prevalence. An arbitrary cut-off was chosen of one group, consisting of those countries with a prevalence of *Campylobacter*-colonised broiler batches below 54.6%, which was the EU median[13] prevalence, and a second group were those countries with a prevalence of

[13] Two non-MSs, Norway and Switzerland, were included to calculate the EU median prevalence.

Campylobacter-colonised broiler batches above the EU median. The results showed that, based on the full models, there were no differences in identified associated factors for these two groups of countries nor in the direction of the effect of those factors or their categories (Appendix D, Table 28). For the lower prevalence country group, the proportion of unexplained variance (in *Campylobacter*-colonisation results) attributable to uninvestigated slaughterhouse-specific factors was 5% whereas for the higher prevalence group of countries it was 40% (Table 5). This indicated that the unexplained variance due to uninvestigated slaughterhouse-specific factors was bigger for the group of countries with prevalence above the EU median compared to the group of countries with prevalence below the EU median.

5.1.2. Analysis of factors associated with *Campylobacter coli*-colonised broiler batches

A full random-effect model investigating factors associated with *C. coli*-colonised broiler batches was fitted including all the available variables: country, flock production type, previous thinning in the flock, age of broilers, quarter of sampling, time (hour) of sampling, and time in hours between sampling and testing. For flock production type, two different codifications were considered. The first was with four categories: conventional, free-range standard, free-range organic and unknown. The second codification merged the categories free-range standard and free-range organic into a unique category. The *P*-values for flock production type (using both codifications), previous thinning in the flock, time (hour) of sampling during the day and time in hours between sampling and testing were not significant; hence they were discarded from the model based on the backward procedure.

The following potential risk factors for *C. coli*-colonised broiler batches were retained in the final logistic mixed-effects model:

- age of the broilers (at sampling);
- quarter of sampling.

The OR estimates for the factors in the final model at EU level are presented in Table 29 in Appendix E. The final model included country-specific effects and the factor 'time (in hours) between sampling and testing' that related to the sensitivity of the testing process (effects not shown). A longer delay increased the risk of detecting *C. coli* from the batches. ORs are adjusted for effects of both factors.

According to the analyses, batches of older birds were more at risk of being colonised with *C. coli*; the risk for positivity increasing in increments of 30% for every increase of 10 days in the age of the slaughtered broilers. Furthermore, the risk of a broiler batch being colonised with *C. coli* was highest in the third quarter (July-September) and lowest in the first quarter (January-March).

The variance of the random effect (effect of slaughterhouses) in the final regression model was significantly positive, meaning that the estimate was much larger than its standard error (Table 30, Appendix E). This indicated that the baseline risk of *C. coli* colonisation of slaughter batches varied between slaughterhouses, even when accounting for other factors i.e. age of broilers, quarter of sampling and the factor 'country'. Consequently, within countries, there were slaughterhouses with an overall higher prevalence and slaughterhouses with an overall lower prevalence of *C. coli*-colonised batches. The proportion of variance (in *C. coli*-colonisation results) that remained unexplained by the investigated factors and that was due to between-slaughterhouse variability, was 20% (Table 5).

Table 5. **Variance components of the final logistic mixed-effects models for factors associated with *Campylobacter*-colonised broiler batches and/or *Campylobacter*-contaminated carcasses, in the EU*, 2008**

Final logistic mixed-effects models	Proportion (%) of unexplained variance attributable to slaughterhouse effects
***Campylobacter* colonisation of broiler batches**	25
Country group with prevalence below the EU median	5
Country group with prevalence above the EU median	40
***Campylobacter coli* colonisation of broiler batches**	20
***Campylobacter* contamination of broiler carcasses:**	
Non-colonised broiler batches	18
Colonised broiler batches	50
Country group with prevalence below the EU median:	
Non-colonised broiler batches	4
Colonised broiler batches	22
Country group with prevalence above the EU median:	
Non-colonised broiler batches	35
Colonised broiler batches	70

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

5.1.3. Analysis of factors associated with *Campylobacter*-contaminated broiler carcasses

A univariate description and bivariate association of factors potentially associated with *Campylobacter*-contaminated broiler carcasses is presented in Appendix F (Tables 31-50 and Figures 20-35). The most relevant information is also displayed in the following.

5.1.3.1. Descriptive analysis of factors potentially associated with *Campylobacter*-contaminated broiler carcasses

5.1.3.1.1. Age of broilers

Figure 4 displays the boxplot of the age of broilers (in days) according to the *Campylobacter*-contamination results of the broiler carcasses. The median age of broilers in the batch from which the carcass originated was slightly greater for *Campylobacter*-positive broiler carcasses than negative carcasses. There are also a considerable number of outliers in the ages observed for both groups of *Campylobacter*-positive and -negative broiler carcasses.

5.1.3.1.2. Quarter of sampling

Similar to the results for *Campylobacter*-colonised broiler batches, the prevalence of *Campylobacter*-contaminated broiler carcasses was highest in the summer period: July, August and September (third quarter), and lowest in the first three months of the year (first quarter) (Figure 5).

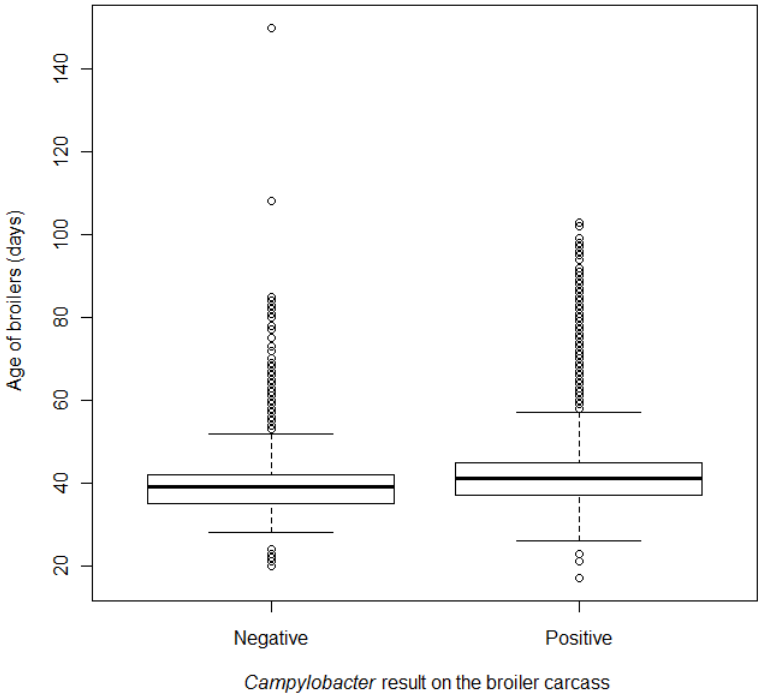

Figure 4. **Boxplot of the age of broiler by *Campylobacter* result on the broiler carcass in the EU*, 2008**

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

In the boxplots, the bottom section of the box represents the first quartile of the distribution and the top section of the box the third quartile, whereas the bar inside the box represents the median. Small circular symbols indicate extreme values, differing from the box > 1.5times the difference between the third and the first quartile (interquartile range).

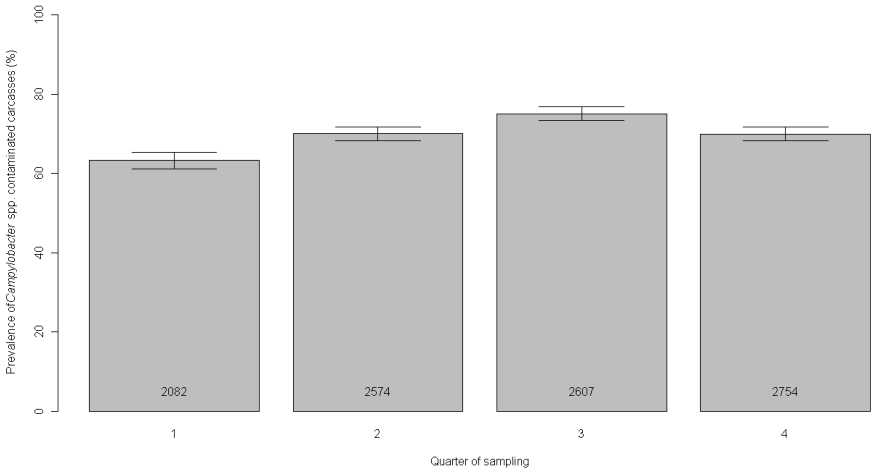

Figure 5. **Prevalence of *Campylobacter*-contaminated broiler carcasses by quarter of sampling in the EU*, 2008**

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

5.1.3.1.3. Time (hour) of sampling during the day

Figure 6 depicts the prevalence for *Campylobacter*-contaminated broiler carcasses according to the time (hour) of sampling during the day. The EU level prevalence of contaminated carcasses increased slightly with the hour of sampling.

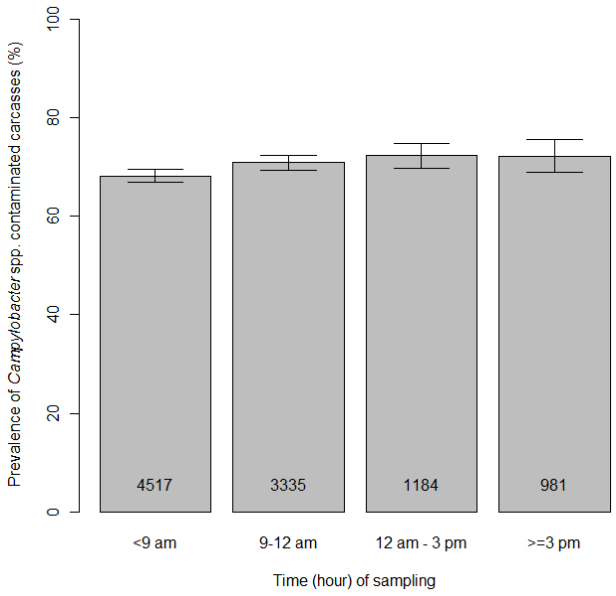

Figure 6. **Prevalence of *Campylobacter*-contaminated broiler carcasses by time (hour) of sampling during the day in the EU*, 2008**

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

5.1.3.1.4. *Campylobacter*-colonisation result in the broiler batch

Figure 7 displays the prevalence of *Campylobacter*-contaminated carcasses according to the *Campylobacter* result in the corresponding broiler batches. The figure indicates that EU level prevalence of *Campylobacter*-contaminated carcasses is much larger when the batch of origin of the carcass was colonised with *Campylobacter* compared to non-colonised batches. This means that there was an association between *Campylobacter*-contamination results on the broiler carcasses and the *Campylobacter*-colonisation results in the broiler batches.

5.1.3.2. Analysis of multicollinearity among potentially associated factors related to *Campylobacter*-contaminated broiler carcasses

The VIF values calculated for the multicollinearity analysis among the factors associated with *Campylobacter* contamination on broiler carcasses (flock production type, previous thinning in the flock, age of broilers, quarter of sampling, time (hour) of sampling, hours between sampling and testing, capacity of slaughterhouse, type of chilling, *Campylobacter*-colonisation result in the broiler batch and *Salmonella*-contamination result on the broiler carcass) are shown in Table 51 (Appendix G). This analysis showed that multicollinearity was not important for the full model since all the VIF values were very small.

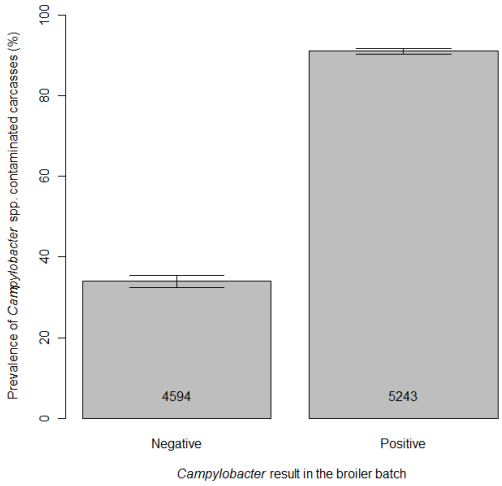

Figure 7. **Prevalence of *Campylobacter*-contaminated broiler carcasses by *Campylobacter*-colonisation result in the broiler batch [(a)] in the EU[*], 2008**

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

(a): A total of 180 samples were excluded from this analysis, as the *Campylobacter* result on contaminated broiler carcasses was missing.

5.1.3.3. Identification of factors potentially associated with *Campylobacter*-contaminated broiler carcasses

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

The variables that were discarded based on the backward procedure were, consecutively: flock production type, previous thinning in the flock, capacity of slaughterhouse, type of chilling of the carcass, and *Salmonella*-contamination result on the broiler carcass.

The following potential risk factors for *Campylobacter*-contaminated broiler carcasses were retained in the final logistic mixed-effects model:

- age of the broilers of the slaughter batch;
- quarter of sampling;
- time of sampling during the day; and
- *Campylobacter* colonisation status of the batch.

The OR estimates for the factors in the final model at EU level are presented in Table 6.

Table 6. **Final logistic mixed-effects model [(a)] for factors associated with *Campylobacter*-contaminated broiler carcasses based on the combined detection and enumeration result, in the EU, 2008**

Factor	Level	Odds ratio*	95% CI		*P*-value
Age of broilers (scale 10 days)		1.26	1.06	1.50	0.0086
Quarter of sampling	*IV*	1.55	1.14	2.11	0.0207
Reference category: *I*	*III*	1.58	1.14	2.19	
	II	1.36	1.02	1.81	
Time (hour) of sampling during the day (scale 1 hour)		1.04	1.00	1.07	0.0366
Campylobacter-colonisation result in the broiler batch	*Yes*	28.62	20.39	40.17	<0.0001
Reference category: *No*					

(a): Estimates and standard errors were assessed using a mixed-effects model with the effect of slaughterhouses included as a random effect (random intercepts) ; with a random effect on the slope of the factor "*Campylobacter*-colonisation result in the broiler batch", and with the factor 'country' included as a fixed effect.

* All ORs were adjusted for the factors 'country' and 'time in hours between sampling and testing', which were both retained in the final model.

The final model included country-specific effects and the factor 'time (in hours) between sampling and testing' that related to the sensitivity of the testing process (effects not shown). A longer delay increased the risk of detecting *Campylobacter* from the carcass samples. ORs are adjusted for effects of both factors.

According to the analyses, the risk of *Campylobacter* contamination of carcasses increased as the age of the broilers in the slaughter batch increased, the risk of positivity increasing in increments of 25% for every increase of 10 days in the age of the slaughtered broilers. Secondly, the risk of having a *Campylobacter*-contaminated broiler carcass was higher in the second, third and fourth quarters of the year compared to the first (January-March). The highest risk for contamination was in the third quarter (July to September). However, the odds values observed for the quarters were lower than in the case of colonisation of broiler batches. Thirdly, the carcasses were also more likely to be contaminated later during the working day i.e. with increasing time (hour) of sampling. Lastly, a *Campylobacter*-colonised batch was approximately 30 times more likely to result in a carcass that is contaminated with *Campylobacter* than a non-colonised batch.

In the final regression model, the variance of the random effects (specific effects of slaughterhouses) was significantly different from zero (*P*-value <0.001, Appendix G, Table 52). This indicated that the baseline risk of *Campylobacter* carcass-contamination varied between the slaughterhouses, even when accounting for other factors i.e. age of the broilers, quarter of sampling, time of processing during the day, *Campylobacter* colonisation and the factor 'country'. Moreover, the variance of the random slopes was significantly different from zero (*P*-value <0.001, Appendix G, Table 52). This also indicated that the risk of obtaining a contaminated carcass from a *Campylobacter*-colonised batch differed between slaughterhouses, within countries. In a similar way, the risk of contaminating a carcass from a non-colonised batch depended on the slaughterhouse. Additionally, the significantly positive covariance between the random intercepts and the random slopes suggested that slaughterhouses characterised by a greater baseline risk of *Campylobacter* contamination of carcasses (higher intercept) tended to have a stronger association between the contamination of carcasses and the colonisation of batches (higher slope of the factor '*Campylobacter*-colonisation result in the broiler batch').

In the case of *Campylobacter*-non-colonised broiler batches, the proportion of unexplained variance (in *Campylobacter*-contamination results) attributable to slaughterhouse-specific factors was 18% (Table 5). Thus, about one-fifth of the unexplained variance was attributable to slaughterhouse-specific factors for which no data were gathered during the survey. In the case of *Campylobacter*-colonised broiler batches, this proportion was 50%, indicating that the unexplained variance due to uninvestigated slaughterhouse-specific factors was bigger when batches were colonised with *Campylobacter*.

The variability between slaughterhouses, with respect to random effects (random intercepts and random slopes), and as estimated by the final model for *Campylobacter* contamination of carcasses is displayed in Figure 8. Notably, the scatterplot visualises for every slaughterhouse two estimates of the following parameters based on the final logistic mixed-effects model:

- the slaughterhouse-specific baseline effect, which is the sum of the country-specific (fixed) effect and the slaughterhouse-specific effect (the random intercept), while keeping the other factors at a fixed level, and
- the slaughterhouse-specific effect of the factor *Campylobacter* colonisation of the broiler batch (the random slope).

In Figure 8, each slaughterhouse is marked either with a dot or triangle. The slaughterhouses located above the line have over 50% prevalence of *Campylobacter*-contaminated broiler carcasses and the slaughterhouses having a prevalence of *Campylobacter*-contaminated broiler carcasses of less than 50% are below the line. Several scenarios can be observed for slaughterhouses with an incoming prevalence of *Campylobacter*-colonised broiler batches below 50% (marked with triangles); some have a prevalence of *Campylobacter*-contaminated broiler carcasses below 50%, while others may have a prevalence at carcass level above or equal to 50%. This means that slaughterhouses that have incoming prevalence of *Campylobacter*-colonised slaughter batches below 50% may generate prevalence of *Campylobacter*-contaminated broiler carcasses below or above 50%, respectively. For slaughterhouses with an incoming prevalence of *Campylobacter*-colonised broiler batches above 50% (marked with dots) the same three scenarios are also observed; some have a prevalence of *Campylobacter*-contaminated broiler carcasses below 50%, while others may have a prevalence at carcass level above or equal to 50%. Thus, other slaughterhouses that have an incoming prevalence of *Campylobacter*-colonised slaughter batches above 50% may generate a prevalence of *Campylobacter*-contaminated broiler carcasses below or above 50%, respectively.

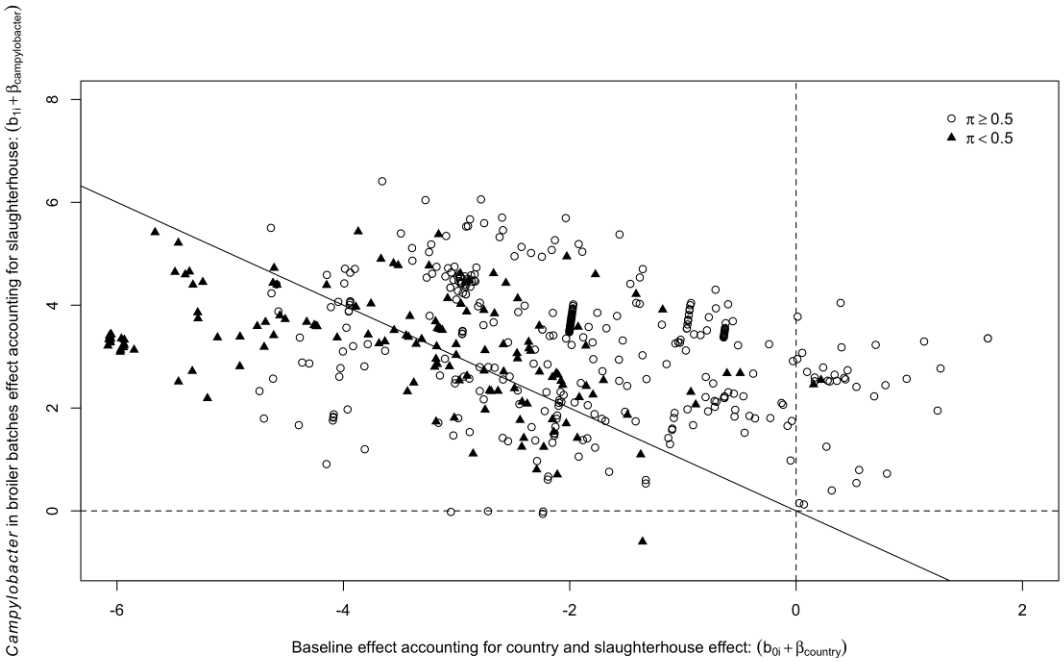

Figure 8. **Scatterplot of the baseline effect (accounting for the fixed effect of country and the random effect of slaughterhouse, i.e. random intercepts) by *Campylobacter* result in broiler batches effect (accounting for the random effect of slaughterhouse, i.e. random slopes)***

* Dots stand for slaughterhouses with a prevalence of *Campylobacter*-contaminated batches above 50% and triangles stand for slaughterhouses below 50%, while a line stands for values of the random intercept and a slope results in a prevalence of *Campylobacter*-contaminated broiler carcasses of 50%.

An additional complementary analysis, following the above modelling approach, was made to compare the full model results for *Campylobacter*-contaminated broiler carcasses between two groups of countries. The arbitrary cut-off was chosen of one group consisting of those countries with a prevalence of *Campylobacter*-contaminated broiler carcasses below 62.5%, which was the EU median[14] prevalence, and a second group were those countries with a prevalence of *Campylobacter*-contaminated broiler carcasses above the EU median. The results are displayed in Appendix H, Table 53.

For the group of countries with a prevalence below 62.5%, the only potential risk factor for *Campylobacter*-contaminated broiler carcasses, that was statistically significant in the full logistic mixed-effects model, was the *Campylobacter* colonisation status of the batch. A *Campylobacter*-colonised batch was approximately 15 times more likely to result in a carcass that was contaminated with *Campylobacter* than a non-colonised batch. However, since this model was not the final one, the effect size is only indicative. In the case of *Campylobacter*-non-colonised broiler batches, the proportion of unexplained variance (in *Campylobacter*-contamination results) attributable to slaughterhouse-specific factors was 4% (Table 5). In the case of *Campylobacter*-colonised broiler batches, this proportion was 22%, indicating that the unexplained variance due to uninvestigated slaughterhouse level factors impacting on the risk of contamination of broiler carcasses was bigger when batches were colonised with *Campylobacter*.

[14] Two non-MSs, Norway and Switzerland, were included to calculate the EU median prevalence.

For the group of countries with a prevalence above 62.5%, the following potential risk factors were statistically significant in the full logistic mixed-effects model for *Campylobacter*-contaminated broiler carcasses:

- flock production type;
- age of slaughter batch broilers;
- quarter of sampling;
- type of carcass chilling;
- *Campylobacter* colonisation status of the batch.

The results displayed in Table 53 are based on a full – not the final – model; hence the observed effects are only indicative. The risk of *Campylobacter* contamination of carcasses was much higher when broilers originated from organic flocks. The risk also increased as the age of the broilers in the slaughter batch increased. The risk of a *Campylobacter*-contaminated broiler carcass was higher in the third and fourth quarters of the year compared to the first (January-March). The risk was higher when mixed chilling methods were used compared to only air chilling. Also, a *Campylobacter*-colonised batch was approximately 50 times more likely to result in a carcass that was contaminated with *Campylobacter* than a non-colonised batch.

Among the high prevalence countries, in the case of *Campylobacter*-non-colonised broiler batches, the proportion of unexplained variance (in *Campylobacter*-contamination results) attributable to slaughterhouse-specific factors was 35% (Table 5). In the case of *Campylobacter*-colonised broiler batches, this proportion was 70%, indicating that the unexplained variance due to uninvestigated slaughterhouse level factors impacting on the risk of contamination of broiler carcasses was bigger when batches were colonised with *Campylobacter*. Also, these proportions were larger compared to the effects in the lower prevalence country group.

5.1.4. Analysis of factors associated with counts of *Campylobacter* on *Campylobacter*-contaminated broiler carcasses

A descriptive analysis of the factors potentially associated with counts of *Campylobacter* on *Campylobacter*-contaminated broiler carcasses is included in Appendix I (Tables 54-63 and Figures 36-44). The most important findings are presented in the following.

5.1.4.1. Descriptive analysis of factors potentially associated with counts of *Campylobacter* on *Campylobacter*-contaminated broiler carcasses

5.1.4.1.1. *Campylobacter*-colonisation result of the broiler batch

Figure 9 displays the boxplot of the $\log_{10}$ *Campylobacter* counts on the broiler carcass according to the *Campylobacter*-colonisation result of the broiler batch. The figure indicates that *Campylobacter* colonisation of broiler batches is associated with positive counts on the carcasses. Figure 10 displays in more detail at EU level the (categorised) *Campylobacter* counts (cfu/g) on broiler carcasses according to whether the batch was colonised, or not, with *Campylobacter*. Most of the *Campylobacter*-non-colonised slaughter batches (approximately 80%) had counts of less than 10 cfu/g. However, in some *Campylobacter*-non-colonised slaughter batches the *Campylobacter* counts on broiler carcasses were distributed among all the defined positive counts categories. About 10% of non-colonised batches had carcasses with counts in the highest level count category, above 10,000 cfu/g. Conversely, for *Campylobacter*-colonised slaughter batches, only about 20% had *Campylobacter* counts on broiler carcasses below 10 cfu/g. The proportion of colonised batches with counts in categories above 10 cfu/g, was for every category, much higher compared to the non-

colonised batches. About 90% of colonised batches had carcasses with counts in the highest level count category, above 10,000 cfu/g.

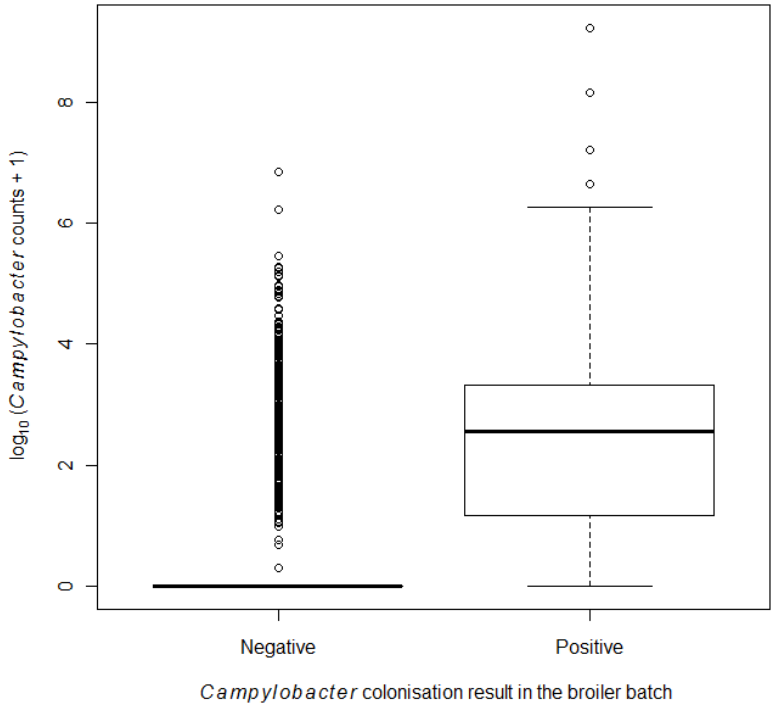

Figure 9. **Boxplot of the log_{10} transformation of '*Campylobacter* counts on broiler carcasses added to 1', by *Campylobacter*-colonisation result in the broiler batch**

* Exceptionally in Luxembourg no *Campylobacter* enumeration was executed in broiler carcass samples, Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

In the boxplots, the bottom section of the box represents the first quartile of the distribution and the top section of the box the third quartile, 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). *Campylobacter* counts were added to one previous to log_{10} transformation in order to allow for the inclusion of negative counts (<10 cfu/g) in these representations.

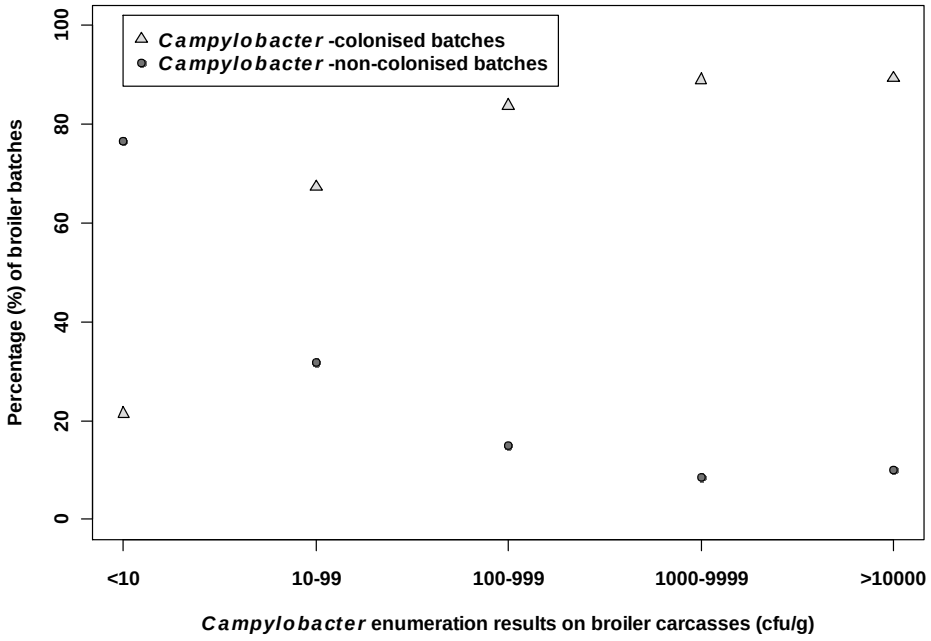

Figure 10. **Categorised *Campylobacter* counts (cfu/g) on broiler carcasses in *Campylobacter*-colonised and -non-colonised broiler batches in the EU, 2008**

5.1.4.2. Analysis of multicollinearity among potentially associated factors related to counts of *Campylobacter* on *Campylobacter*-contaminated broiler carcasses

The calculated VIF values resulting from the multicollinearity analysis shown in Table 51 (Appendix G) apply, as the same factors were considered as being potentially associated with the counts of *Campylobacter* on *Campylobacter*-contaminated carcasses, namely flock production type, previous thinning in the flock, age of broilers, quarter of sampling, time (hour) of sampling, hours between sampling and testing, capacity of slaughterhouse, type of chilling, *Campylobacter*-colonisation result of the broiler batch and *Salmonella*-contamination result on the broiler carcass. This analysis showed that multicollinearity was not important for the full model since all VIF values were very small.

5.1.4.3. Identification of factors potentially associated with counts of *Campylobacter* on *Campylobacter*-contaminated broiler carcasses

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

The factors that were discarded based on the backward procedure were, consecutively: quarter of sampling, age of broilers, flock production type, time of sampling during the day, previous thinning in the flock of origin, *Salmonella* result in broiler batches, type of chilling, capacity of slaughterhouse and time between sampling and testing. All these factors had a *P*-value higher than 0.1. The only

potential risk factor for higher counts of *Campylobacter* on *Campylobacter*-contaminated broiler carcasses that was retained in the final model was the *Campylobacter* colonisation status of the batch, the estimate of which in the final model at EU level is presented in Table 7.

Table 7. **Final model [(a)] for *Campylobacter* counts greater than or equal to 1 cfu/g on broiler carcasses, in the EU, 2008**

Variable	Estimate	Standard Error	*P*-value
Campylobacter-colonisation results of the broiler batch	1.05	0.23	<0.0001

(a): Estimates and standard errors were assessed using a mixed-effects model with the effect of slaughterhouses included as a random effect (random intercepts) and with the factor 'country' included as a fixed effect.

The final model also included the factor 'country' and country-specific effects are presented in Appendix K, Table 64. Table 8 displays the estimate of the variance of the random intercepts (specific-slaughterhouse effects), which was significantly positive (Wald test statistic: 84.4, *P*-value < 0.001). This indicated that there was significant variation between the slaughterhouses in the baseline risk for higher *Campylobacter* counts on *Campylobacter*-contaminated broiler carcasses, even when the other factors *Campylobacter* colonisation status of the batch and 'country' were accounted for. A Likelihood Ratio Test investigating the need of a random intercept was 1,532.32 (*P*-value <0.0001), justifying the mixed-effects model approach.

In addition Table 8 presents the estimate of the overdispersion parameter and its standard error. This parameter was also significantly positive, indicating that the data were overdispersed meaning that the counts were not randomly distributed over the categories. This justified the need to use a negative binomial mixed-effects model – rather than a Poisson regression model.

Table 8. **Final model for *Campylobacter* counts greater than or equal to 1 cfu/g on broiler carcasses: overdispersion parameter and variance of the slaughterhouse-specific random intercepts, in the EU, 2008**

	Estimate	Standard Error	*P*-value
Overdispersion parameter	2.36	0.25	-
Variance of slaughterhouse random intercepts	1.47	0.16	< 0.001

5.2. Descriptive investigation of the *Campylobacter* detection and enumeration methods results of broiler carcass samples

The country-specific data as regards *Campylobacter* detection and enumeration methods test results of broiler carcass samples are presented in Table 9. In this table, ranking is based firstly on the percentage of broiler carcass samples that were found positive by the enumeration test only and secondly on the percentage of broiler carcass samples that were found positive by the detection test only. At EU level, detection of *Campylobacter* from the carcasses was 55.5% [(4,843+713)/10,004] by the detection method, and 51.6% [(4,843+318)/10,004] by the enumeration method. Combining positive results from both culture methods indicated proportion-positive carcasses of 58.7% [(4,843+713+318)/10,004].

Ten countries reported at least one *Campylobacter*-contaminated carcass based on a positive result to the enumeration method only. This specific proportion 'positive by the enumeration method only' ranged from a (very low, below 1%) minimum of 0.5% (Switzerland and Lithuania) to a (high, above 20%) maximum of 35.3% (Belgium). For the United Kingdom, Ireland, Austria, Italy and Germany this proportion was low (between 1% and 10%) whereas for Portugal and Netherlands it was moderate (between 10% and 20%). Overall, 5.4% [318/(4,843+713+318)] of *Campylobacter*-contaminated carcass samples were tested positive by the enumeration method only. Also the reverse situation was encountered and 21 countries reported at least one *Campylobacter*-contaminated carcass based on a positive result to the detection method only. This specific proportion 'positive by detection method only' ranged from a minimum of 0.3% (Malta) to a maximum of 19.2% (United Kingdom). For Romania this proportion was very low (below 1%); for 11 MSs this proportion was low (between 1% and 10%) whereas for the remaining eight MSs (including the United Kingdom) it was moderate (between 10% and 20%). Overall, 12.1% [713/(4,843+713+318)] of *Campylobacter*-contaminated samples were tested positive by the detection method only.

Table 9. **Overview of the *Campylobacter* detection and enumeration methods test results, on broiler carcass samples, in the EU[*], 2008[**]**

Country	Carcass samples	Positives to both detection or enumeration[(a)] methods		Positives to both detection and enumeration methods		Positives to detection method only		Positives to enumeration method only	
	N	N	%	N	%	N	%	N	%
Belgium	380	198	52.1	58	15.3	6	1.6	134	35.3
Netherlands	429	162	37.8	94	21.9	16	3.7	52	12.1
Portugal	421	312	74.1	237	56.3	25	5.9	50	11.9
Germany	432	268	62.0	155	35.9	82	19.0	31	7.2
Italy	393	205	52.2	124	31.6	58	14.8	23	5.9
Austria	408	329	80.6	253	62.0	67	16.4	9	2.2
Ireland	394	386	98.0	378	95.9	0	0	8	2.0
United Kingdom	401	350	87.3	266	66.3	77	19.2	7	1.7
Lithuania	374	172	46.0	170	45.5	0	0	2	0.5
Switzerland	408	288	70.6	210	51.5	76	18.6	2	0.5
Latvia	122	41	33.6	41	33.6	0	0	0	0
Norway	396	20	5.1	20	5.1	0	0	0	0
Slovenia	413	333	80.6	333	80.6	0	0	0	0
Spain	389	360	92.5	360	92.5	0	0	0	0
Malta	367	348	94.8	347	94.6	1	0.3	0	0
Romania	357	227	63.6	225	63.0	2	0.6	0	0
Estonia	102	5	4.9	2	2.0	3	2.9	0	0
Bulgaria	280	126	45.0	117	41.8	9	3.2	0	0
Finland	369	21	5.7	8	2.2	13	3.5	0	0
Poland	419	339	80.9	322	76.8	17	4.1	0	0
Sweden	410	55	13.4	37	9.0	18	4.4	0	0
Slovakia	422	315	74.6	290	68.7	25	5.9	0	0
Hungary	321	180	56.1	160	49.8	20	6.2	0	0
Denmark	396	123	31.1	94	23.7	29	7.3	0	0
Cyprus	357	46	12.9	5	1.4	41	11.5	0	0
France	422	370	87.7	320	75.8	50	11.8	0	0
Czech Republic	422	295	69.9	217	51.4	78	18.5	0	0
EU [(b)]	**10,004**	**5,874**	**58.7**	**4,843**	**48.4**	**713**	**7.1**	**318**	**3.2**

* No analysis for Luxembourg was included as Luxembourg did not perform *Campylobacter* enumeration.

** Countries are presented in this table ranked based firstly on the percentage of broiler carcass samples that were found positive by the enumeration test only and secondly on the percentage of broiler carcass samples that were found positive by the detection test only.

(a): Results of the enumeration method were coded as positive or negative according to whether the *Campylobacter* counts were at least 10 or below 10 cfu/g on contaminated broiler carcasses, respectively.

(b): Including Norway and Switzerland.

5.3. Comparison of the notification rates of campylobacteriosis in humans and the *Campylobacter* prevalence in broiler batches and on broiler carcasses across the EU

Table 10 displays the human notification rates of confirmed campylobacteriosis cases per 100,000 population in 2008 (Community Summary Report on Zoonoses in 2008, EFSA, 2010c). The variation in the notification rates of campylobacteriosis cases among reporting MSs is large and the different sensitivities of the reporting systems and microbiological methods employed by MSs may have influenced these figures; consequently comparison between countries should be carried out with caution. Moreover, for overview purposes only, Table 10 also shows the country-specific prevalence of *Campylobacter*-colonised broiler batches and of *Campylobacter*-contaminated broiler carcasses.

5.4. Analysis of the *Campylobacter* spp. frequency distribution across the EU

Comparisons of the proportion of *C. jejuni* and *C. coli* isolates originating from *Campylobacter*-colonised broiler batches and *Campylobacter* positive broiler carcasses categorised by production type are given in Tables 11 and 12 respectively. This data shows that for broiler batches reared by both free-range production systems (organic and standard) the proportion of *Campylobacter* isolates, which were *C. coli*, was 8% to 14% higher than for broiler batches reared by the conventional production system. A higher contamination percentage of *C. coli* was also observed for broiler carcasses originating from batches reared by the free-range production system alone. However, the differences in the percentages were somewhat smaller (7% to 12%) than for broiler batches.

Table 10. **Comparison between the notification rate of human campylobacteriosis cases and the prevalence of *Campylobacter*-colonised broiler batches and *Campylobacter*-contaminated broiler carcasses, in the EU*, 2008**

Country	2008 Human notification rate (confirmed cases per 100,000 population)[(a)]	Prevalence of *Campylobacter*-colonised broiler batches	Prevalence of *Campylobacter*-contaminated broiler carcasses
Austria	51.4	52.9	80.6
Belgium	47.9	31.0	52.7
Bulgaria	0.2	29.6	45.2
Cyprus	2.9	30.6	14.1
Czech Republic	193.3	61.3	68.6
Denmark	63.4	19.0	31.4
Estonia	11.5	2.0	4.9
Finland	84.0	3.9	5.5
France	5.4	76.1	88.7
Germany	78.7	48.9	60.8
Hungary	54.9	50.1	55.3
Ireland	39.8	83.1	98.3
Italy	0.4	63.3	49.6
Latvia	0	41.0	33.6
Lithuania	22.6	41.5	45.8
Luxembourg	90.7	100	100
Malta	18.8	96.8	94.3
Netherlands	39.2	24.4	37.6
Poland	0.7	78.9	80.4
Romania	<0.1	77.0	64.2
Slovakia	56.7	73.6	79.1
Slovenia	4.3	78.2	77.8
Spain	11.4	88.0	92.6
Sweden	83.8	13.2	14.6
United Kingdom	90.9	75.3	86.3
Switzerland	102.3	59.0	71.7
Norway	60.7	3.2	5.1

* Greece and Portugal did not provide data on the human cases.

(a): (EFSA, 2010c)

Table 11. **Frequency distribution of *C. jejuni* and *C. coli* isolated from *Campylobacter*-colonised broiler batches, per flock production type, in the EU, 2008***

Production type	*C. jejuni*		*C. coli*		Total	
	No of isolates	%	No of isolates	%	No of isolates	%
Conventional	3,006	**60.4**	1,973	**39.6**	4,979	**100**
Free-range standard[(a)]	152	**46.5**	175	**53.5**	327	**100**
Free-range organic[(b)]	35	**52.2**	32	**47.8**	67	**100**

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

(a): Only data from 15 MSs (Belgium, Bulgaria, Estonia, France, Hungary, Ireland, Italy, Luxembourg, the Netherlands, Poland, Portugal, Romania, Slovakia, Spain and the United Kingdom) and one non-MS (Switzerland) reporting *C. jejuni*- and/or *C. coli*-colonised broiler batches originating from free-range standard production systems, were taken into account. Estonia reported all flocks as free-range standard, but broilers were kept inside with no access to the outside and these data are included in the analysis.

(b): Only data from 10 MSs (Austria, Belgium, Bulgaria, France, Germany, Ireland, Italy, the Netherlands, Portugal and the United Kingdom) and one non-MS (Switzerland) reporting *C. jejuni*-and/or *C. coli*-colonised broiler batches originating from free-range organic production systems, were taken into account.

Table 12. **Frequency distribution of *C. jejuni* and *C. coli* isolated from *Campylobacter*-contaminated broiler carcasses, per flock production type, in the EU, 2008***

Production type	*C. jejuni*		*C. coli*		Total	
	No of isolates	%	No of isolates	%	No of isolates	%
Conventional	3,545	**64.0**	1,996	**36.0**	5,541	**100**
Free-range standard [(a)]	193	**52.4**	175	**47.6**	368	**100**
Free-range organic [(b)]	43	**57.3**	32	**42.7**	75	**100**

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

(a): Only data from 15 MSs (Belgium, Bulgaria, Estonia, France, Hungary, Ireland, Lithuania, Luxembourg, the Netherlands, Poland, Portugal, Romania, Slovakia, Spain and the United Kingdom) and one non-MS (Switzerland) reporting *C. jejuni*- and/or *C. coli*-positive broiler carcasses originating from free-range standard production systems, were taken into account. Estonia reported all flocks as free-range standard, but broilers were kept inside with no access to the outside and these data are included in the analysis.

(b): Only data from 10 MSs (Austria, Belgium, Bulgaria, France, Germany, Ireland, Italy, the Netherlands, Portugal and the United Kingdom) and one non-MS (Switzerland) reporting *C. jejuni*-and/or *C. coli*-positive broiler carcasses originating from free-range organic production systems, were taken into account.

From Tables 11 and 12 it can be observed that, when the slaughtered broilers or their carcasses originated from conventional flock production types, the proportion of *C. jejuni* isolates out of the total speciated isolates was about two-thirds, and *C. coli* isolates about one-third. For free-range standard and organic flocks, the distributions of *C. jejuni* and *C. coli* isolates were about equal for each species. Therefore, it seems that relatively more isolates were speciated as *C. coli* in free-range or organic flocks. Appendix L (Figures 45-48) includes maps with the species-specific prevalence distribution in the EU for *C. coli* and *C. jejuni* in broiler batches and on broiler carcasses. This *Campylobacter* spp. distribution in broiler batches and on broilers carcasses across the EU and the two non-MSs was very variable. In the northern countries (Norway, Sweden, Finland and Estonia) only *C. jejuni* were isolated, whereas in all other countries *C. coli* were also detected. In southern EU MSs the presence of *C. coli* was more abundant and in some MSs more than half of the isolates belonged to this species.

6. Discussion

Campylobacter is a known common inhabitant of the caeca of broilers and a high prevalence of colonisation has been reported in the EU-wide baseline survey as previously published in the Part A report on the analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Salmonella* on broiler carcasses in the EU, 2008 (EFSA, 2010a). Broilers can become colonised by *Campylobacter* following exposure to viable bacteria from the environment and presence of *Campylobacter* in the caeca can be at a detectable level after a few hours (Bull et al., 2006). Colonisation of most in-contact birds may take place within a few days of exposure and it may take up to a week for the flock to be fully colonised. Campylobacteriosis remains the most commonly reported food-borne illness in humans in the EU (EFSA, 2009b). The data provided by the Part A report contributed to other evidence, from molecular subtyping and epidemiological studies, identifying poultry meat as an important source of food-borne transmission of human campylobacteriosis. The EFSA Panel on Biological Hazards estimated in its recent scientific opinion on the quantification of the risk posed by broiler meat to human campylobacteriosis cases (EFSA, 2010b) that the handling, preparation and consumption of broiler meat may account for 20% to 30% of human campylobacteriosis cases, while 50% to 80% may be attributed to the chicken (broiler) reservoir as a whole. *Campylobacter* strains from the broiler reservoir may reach humans via routes other than food (e.g. by the environment or by direct contact). The main motivation to control *Campylobacter* in broilers is to protect public health, since *Campylobacter* does not cause clinical disease in poultry.

6.1. Context of *Campylobacter* baseline survey

This EU-wide baseline survey estimated the prevalence of *Campylobacter*-colonised broiler batches and of *Campylobacter*- or *Salmonella*-contaminated broiler carcasses within 26 MSs and two non-MSs and these estimates were published in the Part A report. During the conduct of the survey, some mandatory complementary data were recorded pertaining to the broilers sampled, the slaughterhouse involved in processing and subsequent sample handling. The pragmatic choice as to which potential factor to collect data on was made by MSs, partly based upon EFSA's proposal for the survey design (EFSA, 2007). This Part B report considers whether any of these factors were associated with the presence of *Campylobacter* in broiler slaughter batches or on carcasses. It should be noted that many potential factors of relevance to *Campylobacter* colonisation in slaughter batches or contamination of carcasses, such as –the presence of other farm animals in the holding, hygiene during slaughter and subsequent processing, slaughter techniques, the speed of the slaughter line and the cleaning and disinfection procedures used, were not a part of the present survey. Furthermore, sampling was performed to broadly represent the production methods present in participating countries, so the numbers of broilers sampled from minority production systems was low. For example, 90% of sampled batches originated from conventionally-reared (i.e. housed) birds and about 70% of the chilling methods used for the batches were 'air'. Thus, the baseline survey dataset was unbalanced with respect to certain categories of factors. Consequently, the power of subsequent analyses was hampered by these small amounts of data in those sub-populations, resulting in an inability to eliminate chance as the cause of findings, which is important for the interpretation of the results of the associated factor analyses. As such, the present report does not aspire towards a comprehensive analysis of all risk factors believed to impact on *Campylobacter* risk, but an assessment of, with reasonable confidence, those factors for which information was captured in this survey by a questionnaire-indicated association with *Campylobacter* positivity.

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

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

MSs should consider the information available in this report as an adjunct to the understanding of the nature of the *Campylobacter* problem. These outcomes may inform national control programmes or subsequent in-depth research. The results of this survey are also used in the ongoing quantitative risk assessment of *Campylobacter* in broiler meat by EFSA' Panel on Biological Hazards.

6.2. Analysis of factors associated with *Campylobacter* prevalences

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

Additional analyses performed at the level of the two countries groups in this survey (i.e. MSs having a low or high *Campylobacter* prevalence) should be regarded as a preliminary attempt to investigate effects of reported factors in these countries groups. Moreover, it allowed the assessment of the variability of those effects between these countries groups having different levels of *Campylobacter* colonisation or contamination. The risk factor analyses results at the level of the two countries groups should be regarded as indicative and need to be complemented by specific studies carried out at national level and taking into account domestic conditions.

With these results it is worth considering the interpretation of findings where an association was not found. The statistical methods used are able to provide a robust answer to the question of whether the studied variable is associated with prevalence in this dataset. A statistically significant conclusion from the multivariable regression model, indicates that the outcome of association would have been extremely unlikely to have arisen by chance. In some instances trends of association would appear to be present, but chance occurrence of random events could not be discounted as a cause of the observed trend in this dataset and so the observation is not statistically significant. This absence of a statistically significant result should not be construed to disprove association, merely the inability to rule out chance and therefore the absence of mention in the final model as an associated factor should be interpreted in this context.

6.2.1. Analysis of factors associated with the prevalence of *Campylobacter*-colonised broiler batches

Campylobacter are common inhabitants in the intestinal tracts of many warm-blooded animals. Broilers are *Campylobacter*-free on the day of hatching since no vertical transmission takes place (Callicott et al., 2006). The organism is transferred horizontally, usually from a contaminated environment into the broiler flock (Newell and Fearnley, 2003). The impact of biosecurity measures, preventing *Campylobacter* being transmitted from the environment, is therefore broadly accepted. Many factors have been investigated to estimate risk factors for a broiler flock to be colonised by *Campylobacter*. In the present baseline survey, the following six potentially associated factors were studied: flock production type, previous thinning in the flock, age (days) of broilers at slaughter, quarter (of the year 2008) of sampling, time (hour) of sampling during the day, and time (hours) between sampling and testing.

Besides significant differences between the countries, the analyses of the pooled caecal contents survey results showed that three factors were significantly associated at EU level with *Campylobacter* colonisation of broiler batches. The risk of colonisation increased with the thinning practices in the flock of origin (with slaughter batches of previously thinned flocks being at significantly higher risk of colonisation), with age of the slaughtered broilers and with sampling time during the year (with the

period July-September being the quarter at most risk). These findings correspond with other studies on risk factors for *Campylobacter* in broilers (Berndtson et al., 1996; Bouwknegt et al., 2004; Barrios et al., 2006).

Some farmers apply partial depopulation or "thinning" of flocks for economic and/or welfare reasons, i.e. from within a single flock a subset of birds typically of a specific size are selected and sent for slaughter with the remaining subset allowed to continue growing. When the first group of birds is collected, the biosecurity of housed birds can be breached and *Campylobacter* can be introduced into the flock and the remaining birds may become colonised. Additionally the stressful intrusion into the growing environment may predispose remaining birds to colonisation. The risk with "thinning" has been reported from other studies (Allen et al., 2008; Hald et al., 2001; Wedderkopp et al., 2000), however studies in the Netherlands indicate that this risk is confounded by age (Russa et al., 2005).

Age has previously been identified as a risk factor for *Campylobacter* colonisation in broilers (Bouwknegt et al., 2004). Under natural conditions, *Campylobacter* is rarely found in a broiler flock before two weeks of age. This may be (or is probably) due to maternal antibodies or innate enteric immunological factors. After the second week of life, colonisation easily occurs up to about eight weeks of age, when a reduction in *Campylobacter*-concentration and a decreased proportion of colonised birds have been reported to occur which may be due to acquired immunity (Newell and Fearnley, 2003).

Seasonal variation in *Campylobacter* in broilers, with a peak in the summer has repeatedly been reported from several countries in northern Europe, e.g. Sweden (Hansson et al., 2007), Denmark (Wedderkopp et al., 2000), Norway (Hofshagen and Kruse, 2005), and the Netherlands (Bouwknegt et al., 2004), but has also been reported from France (Refrégier-Petton et al., 2001). In contrast, some studies in the United Kingdom, USA, and Canada have reported no seasonal influence on *Campylobacter* prevalence (Gregory et al., 1997; Humphrey et al., 1993; Nadeau et al., 2002). The reason behind the association of higher *Campylobacter* prevalence and season is not clarified, but has been shown to be temperature-related, notably the effect of ambient temperature on the levels of environmental contamination (Jore et al., 2010). The observation of the seasonal peak might reflect a low background level of prevalence during winter months. Consequently, in countries with a higher background level a seasonal peak would not be easily detected. It has also been suggested that seasonal variation might relate to the abundance of flies (as mechanical vectors) and/or increased ventilation because of higher temperatures during the summer (Hald et al., 2008). Every factor that implies contact with the outdoor environment could be regarded as a risk for introducing *Campylobacter* into the flock.

Additional analyses performed for the two groups of countries, notably countries with a high or low prevalence of *Campylobacter*-colonised broiler batches, respectively, using the median[15] of prevalence (54.6%) as the cut-off point, showed that the same potential risk factors resulted as significant for both categories of countries (thinning, age, and quarter of sampling).

Delaying the time between sampling and testing increased the risk of detecting *Campylobacter* from the broiler batches samples. No obvious explanation for this finding could be found but it might be that laboratory-specific effects confounded these results. This observation however indicates that harmonisation of testing procedures should be considered of importance by MSs when designing national *Campylobacter* control programmes.

The analysis at EU level further indicated that the slaughterhouse-specific baseline risk of colonisation of batches with *Campylobacter*, varied significantly between countries and between slaughterhouses within countries. Thus, there were slaughterhouses (within countries) with higher prevalence and slaughterhouses with lower prevalence of *Campylobacter*-colonised batches.

[15] Two non-MSs, Norway and Switzerland, were included to calculate the EU median prevalence.

Still, slaughterhouse-specific effects on *Campylobacter* colonisation of the intestine are considered minimal although it may happen when *Campylobacter*-contaminated crates are used for the transport of the flock to the slaughterhouse (Hansson et al., 2005). The variation across slaughterhouses might reflect differences of on-farm colonised flocks that are delivered to the slaughterhouses. It could be that on-farm *Campylobacter*-colonised flocks tend to be sent to certain slaughterhouses or the between-slaughterhouse variability might mirror regional differences in on-farm colonised flocks. Still, there may be confounders that have not been identified in the present study. For example, Bouwknegt et al., (2004) have reported an association between prevalence and integrations.

As mentioned above, many potential factors of relevance to *Campylobacter* colonisation in slaughter batches, such as the presence of other farm animals in the holding, were not a part of the present survey. In this context the analyses showed that one fourth of the unexplained variance was attributable to slaughterhouse-specific factors for which no data were gathered during the survey. The magnitude of the unexplained variance due to uninvestigated slaughterhouse-specific factors was bigger for the group of countries with prevalence above the EU median.

6.2.1.1. Analysis of factors associated with the prevalence of *Campylobacter coli*-colonised broiler batches

The most prevalent *Campylobacter* species isolated from broiler batches were *C. jejuni* and *C. coli*, which is in accordance with findings in other international studies. *C. jejuni* was the most frequently isolated species in most countries, however in seven countries *C. coli* dominated.

In the EU level analysis a significant association was found between the age of broilers and *C. coli* colonisation. Previous investigations have considered whether younger chickens are more easily colonised by *C. jejuni* and as they grow older, *C. coli* takes over. This has been reported from a study in the United Kingdom of organic and free-range chickens (El-Shibiny et al., 2005). Also the sampling quarter during the year 2008 put batches at risk of *C. coli* colonisation with the period July-September being the quarter at most risk. No other associations were indicated, including the flock production type e.g. flocks with outdoor access. *Campylobacter* spp. frequency distribution across the EU is discussed in Section 6.4.

C. jejuni and *C. coli* are closely related species, which could be difficult to differentiate between when solely reliant on phenotypic methods for identification. There is practically only one biochemical test, the hippurate hydrolysis test that distinguishes *C. jejuni* from *C. coli*. *C. jejuni* hydrolyses hippurate, but hippurate-negative strains occur and could be falsely identified as *C. coli*. Genotypic techniques such as PCR-based methods are more reliable since they detect DNA sequences unique for the respective species (Best et al., 2003; On and Jordan, 2003). In the baseline survey, both phenotypic and genotypic methods were applied.

6.2.2. Analysis of factors associated with the prevalence of *Campylobacter*-contaminated broiler carcasses

6.2.2.1. Effect of *Campylobacter* colonisation status of the broiler batch on contamination of broiler carcasses

In this survey, *Campylobacter* contamination of the carcass was affected by the *Campylobacter* colonisation status of the broiler batch as reflected by the pooled caecal contents sample. In the EU level multivariable regression analysis, a *Campylobacter*-colonised batch was about 30 times more likely to yield a *Campylobacter*-contaminated carcass. This positive association, albeit strong, can be regarded as an expected finding, since *Campylobacter* is a known common inhabitant of the intestinal tract of broilers. Thus the organism can be expected to contaminate broiler carcasses during the

slaughtering process as a result of faecal contamination. Since broiler carcasses are a food product on which the skin of the animal remains, there also exists potential for carry-over of pre-existing faecal contamination of the skin (e.g. from contaminated faeces on crates during transportation to the slaughterhouse) through the slaughter process.

These EU level analyses results indicated that processing of non-colonised broiler batches strongly reduces the risk of subsequent contamination of carcasses with *Campylobacter*. Therefore, controlling the *Campylobacter* prevalence in broiler flocks during primary production (i.e. from farm to slaughtering) would have a beneficial impact on *Campylobacter* contamination of carcasses and broiler meat. These controls are also likely to reduce the overall *Campylobacter* contamination of the slaughterhouse environment, since incoming broilers are the primary source of *Campylobacter* ingress to slaughterhouses causing *Campylobacter*-negative birds to be contaminated during processing from other colonised flocks via the processing environment. Unlike *Salmonella*, *Campylobacter* does not grow in the slaughterhouse environment, which emphasises the importance of the incoming flock as a source of contamination of the environment. Also, the production of *Campylobacter*-negative flocks would also be expected to reduce the potential non-food-borne routes of transmission from broilers as indicated in EFSA's scientific opinion on the quantification of the risk posed by broiler meat to human campylobacteriosis cases (EFSA, 2010b).

The survey results also underline the role of the slaughterhouse environment in *Campylobacter* carcass contamination. Even though a colonised broiler batch was more likely to yield a contaminated carcass, there were many contaminated carcasses deriving from broiler batches that tested negative. Some of these may be due to the limited testing sensitivity to detect all *Campylobacter*-colonised batches, but others may result from cross-contamination from other carcasses or through contact with contaminated surfaces or equipment within the slaughterhouses (Allen et al., 2007; Berrang and Dickens, 2000; Berrang at al., 2001; Berrang and Dickens, 2004; Berrang et al., 2004; Rosenquist et al., 2006). Consequently, good slaughter hygiene is also vital in the prevention of carcass *Campylobacter* contamination.

6.2.2.2. Effect of the slaughterhouse on the risk of *Campylobacter* contamination of broiler carcasses

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

Furthermore, the analysis showed that, depending on the slaughterhouse, *Campylobacter* colonisation in broiler batches arriving on the slaughter line has either a stronger or weaker impact on carcass contamination. In some slaughterhouses carcasses were more likely to become contaminated with *Campylobacter* than in others, when processing either colonised or non-colonised broiler batches. Apparently certain slaughterhouses were more capable of controlling and preventing *Campylobacter* contamination risk in the slaughter process. This implies that the slaughterhouse and the processing steps offer both an opportunity for *Campylobacter* risk mitigation in broilers and can contribute to increase the risk, notably in the case of poor hygienic performances.

As mentioned above, many potential factors of relevance to the *Campylobacter* contamination of broiler carcasses were not a part of the present survey. Slaughterhouse-specific factors with a potential effect on the risk of *Campylobacter* contamination of carcasses, that might explain the observed heterogeneity between slaughterhouses, could relate to the within-batch *Campylobacter* prevalence in the (incoming) slaughter batches or to the bacterial load of the broiler caeca (in addition to the recorded positive-negative status). Moreover, other slaughterhouse effects might relate to

slaughter hygiene practices impacting on the extent to which caecal and faecal contents contaminate carcasses.

In this context the analyses showed that, in the case of *Campylobacter*-non-colonised broiler batches, about one-fifth of the unexplained variance was attributable to slaughterhouse-specific factors for which no data were gathered during the survey. In the case of *Campylobacter*-colonised broiler batches, this proportion was 50%. This indicates that the unexplained variance due to uninvestigated slaughterhouse-specific factors was bigger when batches were colonised with *Campylobacter*. This between-slaughterhouse variability was also bigger for countries with prevalence above the EU median[16]. Thus, it may be in the interest of MSs to investigate further these uninvestigated slaughterhouse-specific factors in their country in order to improve the control of *Campylobacter* and the protection of public health.

6.2.2.3. Effect of other factors on the risk of *Campylobacter* contamination of broiler carcasses

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

Besides significant differences between the countries, the analyses of the carcass survey results showed that three factors were significantly associated at EU level with *Campylobacter* contamination of broiler carcasses; the risk of contamination with *Campylobacter* increased with the age of the slaughtered broilers, with processing later during the day and with sampling during the year, with the period July-September being the quarter at most risk.

The increasing age of broilers has been identified as a risk factor for *Campylobacter* colonisation, hence older birds are found to be more frequently infected at the time of slaughter (Evans et al., 2000; Hartnett et al., 2001).

The prevalence of *Campylobacter*-contaminated broiler carcasses increased when the carcasses were processed later during the day. A possible explanation could be cross-contamination from *Campylobacter*-colonised batches slaughtered earlier during the same day (Johannessen et al., 2007). This is consistent with environmental accumulation of contamination in the slaughterhouse environment and this indicates the need to investigate the mechanism and relevance, in terms of the level, of any accumulation of contamination as well as the potential efficacy of slaughterhouse-specific hygienic procedures and or other interventions.

Similar to the results for *Campylobacter* in broiler batches, a seasonal effect on the risk of *Campylobacter* contamination of broiler carcasses samples was observed. The highest prevalence corresponds to the summer period and the lowest prevalence corresponds to the first quarter. Delaying the time between sampling and testing increased the risk of detecting *Campylobacter* from the broiler batches samples. No obvious explanation for this finding could be found but it might be that laboratory-specific effects confounded these results. This observation however indicates that harmonisation of testing procedures should be considered of importance by MSs when designing national *Campylobacter* control programmes.

There was no evidence of association between the results of *Campylobacter* and *Salmonella* testing based on broiler carcass samples, despite the fact that both represent faecal contamination of carcasses. This might reflect that the prevalence of *Campylobacter*-contaminated carcasses was not importantly influenced by factors favouring cross-contamination or spread of both bacteria. This

[16] Two non-MSs, Norway and Switzerland, were included to calculate the EU median prevalence.

result probably reflects the different epidemiology and ecology of these organisms, for example differences in transmission routes to the broiler flocks on farm. Therefore it is important to realise that meaningful and effective *Salmonella* control programmes may not necessarily imply benefit when managing *Campylobacter* risk.

At EU level, no significant difference was disclosed between conventional broiler flock production types and the other production types. Other studies have reported flock prevalence of *Campylobacter* to be generally higher in organic and free-range flocks with outdoor access compared to conventionally-reared flocks, due to higher environmental exposure of the birds as well as the increased age of the birds at slaughter (Heuer et al., 2001; Lund et al., 2003; McCrea et al., 2006; Näther et al., 2009). It might be that, as explained above, the power of the EU level analysis was hampered by too few data in certain sub-populations such as non-conventional production flock types, resulting in an inability to eliminate chance as the cause of findings. Indeed, 90% of the sampled batches originated from 'conventional' flock production. It is noteworthy that there were indications that the risk of *Campylobacter* contamination of carcasses was much higher in the case of broilers originating from organic flocks, in the subset of countries with an above 62.5% carcass prevalence.

No association between the *Campylobacter*-contamination result on the broiler carcass and the capacity of the slaughterhouses was observed in this survey. However, several studies have shown that differences in prevalence of *Campylobacter* result from different slaughtering practices (EFSA, 2005; Rosenquist et al., 2006). As reported above, a significant impact of the different process operations, such as scalding, defeathering, evisceration, washing and chilling on the prevalence of *Campylobacter*-contaminated broiler carcasses was indicated. The different slaughter processing steps may contribute to cross-contamination between birds within a flock and between flocks slaughtered successively, or may reduce existing skin contamination to a useful extent. Experience has shown that certain modifications of the processing operation may result in significant reductions in contamination of carcasses with *Campylobacter* species. Thus, the processing procedures seem to be more crucial for the *Campylobacter* contamination of carcasses than the capacity of the slaughterhouses (EFSA, 2005). However, details on the slaughtering process, except the type of chilling, were not collected in this baseline survey because of the wide diversity of processes that could be expected.

Also, EU level analyses did not report an association between the *Campylobacter*-contamination result on the broiler carcass and the type of chilling of carcasses. However, there were indications that the risk of *Campylobacter* contamination of carcasses was higher in the case of broiler carcasses chilled by a mixed-chilling method in the subset of countries with a carcass prevalence above 62.5%. One analytical approach, "the mixed category" grouped the categories combining more than one chilling method and such combined chilling methods, on average, resulted in more cross-contamination/redistribution of *Campylobacter* between carcasses or tended to increase cross-contamination. With regard to the other chilling methods used, as explained above, the power of the EU level analysis could have been hampered by too few data in certain sub-populations such as the use of certain chilling methods, resulting in an inability to eliminate chance as the cause of the findings. Indeed, 70% of the chilling methods used for the batches were 'air'. Still, the likely diverging outcome in factors identified at EU level and country-group level datasets indicated that risk factors for *Campylobacter* contamination of carcasses are likely to change according to prevalence situations, whether low or high. In general, broiler carcasses should be chilled as quickly as possible to limit the growth of microorganisms on the carcasses. Whilst *Campylobacter* will not readily replicate below 25˚C, effective chilling and drying of the carcass appears to decrease the viability of *Campylobacter* present. Types of chilling processes used within the EU, include air, immersion and spray chilling. Chilling of carcasses will often decrease the *Campylobacter* contamination level of carcasses (Rosenquist et al., 2006, Allen et al., 2007). Air and immersion chilling have been compared in several studies and these chilling systems seem to be microbiologically comparable (Huezo et al., 2007). However, the large number of variables makes a true comparison among chilling methods rather difficult. Spray chilling has been shown to be

effective in reducing contamination levels, but may also enhance microbial dispersal (Allen et al., 2007).

6.2.3. Analysis of factors associated with counts of *Campylobacter* on contaminated broiler carcasses

When specifically analysing the *Campylobacter* enumeration results at the EU level, the risk of higher counts of *Campylobacter* on a *Campylobacter*-contaminated broiler carcass was only associated with two factors, notably the *Campylobacter* colonisation of the batch of origin of the carcass and the country. Higher *Campylobacter* counts were more likely to be found on carcasses derived from colonised broiler batches. This result confirms the descriptive finding of the Part A report of a tendency for countries with a higher prevalence of *Campylobacter*-colonised slaughter batches to have higher contamination levels on carcasses. This finding also indicates that cross-contamination, from colonised batches, of broiler carcasses derived from non-colonised batches results on average in lower *Campylobacter* counts (lower contamination levels) on carcasses. Secondly, the risk of higher counts on the broiler carcasses also varied significantly between countries.

No other investigated factor (flock production type, previous thinning in the flock of origin, age of broilers, quarter of sampling, time of sampling during the day, time between sampling and testing, capacity of slaughterhouse, type of chilling and *Salmonella*-contamination result on the broiler carcass) was significantly associated with the risk of having higher *Campylobacte*r counts on the carcass. Whereas the analysis of factors associated with the prevalence of *Campylobacter*-contaminated carcasses was based on the total dataset of 10,004 carcass samples, the dataset covering the analyses factors associated with higher counts of *Campylobacter* on a *Campylobacter*-contaminated broiler carcass was made up of the 5,074 carcass samples that had countable enumeration results of at least 1 cfu/g. These data were also overdispersed, meaning that the data values were not randomly distributed across all categories of counts. These particularities of the dataset may have resulted in the inability to rule out chance and caused the absence of mention of the enumerated factors.

In addition to large differences between countries, the risk of higher counts varied significantly between slaughterhouses within countries indicating that some slaughterhouses were more effective at controlling the counts on the carcasses than others. Few data were collected in this survey about slaughterhouse-specific factors that might relate to the number of *Campylobacter* bacteria (counts) on the carcasses. These factors may include the within-batch *Campylobacter* prevalence or the bacterial load of the broiler caeca in the incoming slaughter batches as well as the slaughter hygiene practices impacting on the extent to which caecal and faecal contents contaminate carcasses. It has been reported that the levels of carcass contamination of broilers from *Campylobacter*-colonised batches may increase or decrease depending on the processing step. Examples of operations that often increase the contamination levels are plucking and evisceration (EFSA, 2005; Rosenquist et al., 2006).

Consumer risk appears to be particularly associated with exposure to high numbers of *Campylobacter* (Lindqvist and Lindblad, 2008; Nauta et al., 2009; EFSA, 2009c) and, therefore, the above survey results further emphasise the crucial importance of measures to prevent or reduce *Campylobacter* infections in broiler flocks in primary production since the high *Campylobacter* counts were strongly associated with the colonisation of the slaughter batch. In addition, the results indicate that it is possible to take measures to control the extent of carcass contamination at the slaughterhouse and to prevent high counts on the carcasses. It is therefore important to identify (in order to develop and implement control) the factors at a slaughterhouse that impact on the load on a carcass.

6.3. Descriptive investigation of *Campylobacter* detection and enumeration methods results of broiler carcass samples

All tests have imperfections, and even within the confines of a harmonised survey protocol there exists the potential for imperfections to be accentuated in some circumstances, e.g. a specific laboratory environment. Also, it is important to mention that only one carcass was collected per slaughter batch for analysis. Since there might have been possible slaughter batch variability, this sampling scheme was not optimal to appreciate aspects of variability, especially related to the enumeration method. As explained in the Part A report (EFSA, 2010a) the enumeration method was by nature less sensitive than the detection method. The detection method has a higher analytical sensitivity, i.e. lower levels of *Campylobacter* are detected. This was observed in this survey and 21 countries reported *Campylobacter*-contaminated carcasses based on a positive result to the detection method only. These results are in line with current knowledge and take into account the difference in inoculum size between the detection and enumeration method and the inability of the enumeration method to count low numbers of bacteria. The country-specific proportions of carcasses tested positive by the detection method only varied and this might mirror different scenarios in countries as regards to the bacterial load of the tested samples. However, 10 countries reported at least one *Campylobacter*-contaminated carcass based on a positive result to the enumeration method only. The country-specific proportions of carcasses tested positive by the enumeration method only varied importantly between the 10 countries and ranged from 0.5% to 35.3%. In these cases it can be concluded that the detection method yielded false negative results indicating that the *Campylobacter* present in the sample had not been able to grow sufficiently in the enrichment media possibly due to growth of other background flora or to inhibitory effects of the enrichment media (Habib et al., 2008; Jasson et al., 2009). For the *Campylobacter* contamination analyses, the combined result for the sample (i.e. detection and enumeration) was deemed positive for the presence of *Campylobacter*. The use of this parallel testing of broiler carcasses by both detection and enumeration methods to determine prevalence has resulted in an increased probability of obtaining positive test results for *Campylobacter* in broiler carcasses than the single examination by either method would have done.

These results suggested that the diagnostic sensitivity of the used detection method may have varied between MSs (or laboratories because generally few or only one laboratory analysed the samples). In the case of Belgium an explanation was found and published for the notably low sensitivity of the detection test (Jasson et al., 2009) referring to the potential of high numbers of extended-spectrum-beta-lactamase (ESBL) producing *Escherichia coli* interfering with the detection of *Campylobacter* spp. and thus detracting from the methods' sensitivity. Whilst the detection method used is the international best practice, and utilises conditions designed to select *Campylobacter* and suppress other organisms, ESBL *E. coli* are resistant to the concentration of sodium cefaperazone used in the Bolton enrichment broth and mCCDA used in the standardised detection methodology and thus may suppress the *Campylobacter* spp. during the execution of the method.

These findings indicate that test imperfections should not be overlooked when interpreting test-based prevalence estimates and are consistent with the results of a recent study by Edson et al. (2009), who presented results from the proficiency testing of *Campylobacter* spp. detection in U.S. food laboratories (ranging from 380 to 442) over the period 1999 to 2007. The cumulative nine-year false-negative rate was 13.6% (and for *C. coli* specifically, was 24.0%), which is considerably higher compared to a rate of 5.9% for *Salmonella* spp. and 7.2% for *Listeria monocytogenes*, as concluded in the same study. This showed that misclassification in the testing of *Campylobacter* was notably higher compared to other food-borne bacteria.

Low detection method test sensitivity implies that the actual true prevalence of *Campylobacter*-contaminated carcasses might be more importantly underestimated for some MSs. Thus, it might be valuable for MSs with a lower than expected (detection method) test sensitivity to analyse more in-depth the national survey results to investigate the factors potentially affecting test sensitivity. Appropriate statistical and modelling tools (e.g. Bayesian statistical modelling) are available to

estimate better the actual national true prevalence of *Campylobacter*-contaminated carcasses. An example for such a modelling approach was previously used in the case of *Campylobacter* in the broiler sector in the Netherlands and Belgium (Woldemariam et al., 2008; Habib et al., 2008). Also, the possible detection method test bias warrants caution while interpreting the results of the EU level analyses of factors associated with the prevalence of *Campylobacter*-contaminated broiler carcasses or with the prevalence of *Campylobacter*-colonised broiler batches. In conclusion, surveys based on diagnostic test results will always be concealed by the inaccuracy of those diagnostic tests and this cannot be solved by a complex analysis.

6.4. *Campylobacter* spp. frequency distribution across the EU

C. jejuni, *C. coli* and *C. lari* are three potentially food-borne species within the thermophilic species in the genus *Campylobacter*. The *Campylobacter* spp. distribution in broiler batches and on broilers carcasses across the EU and the two non-MSs was highly variable. In the northern countries (Norway, Sweden, Finland and Estonia) only *C. jejuni* were isolated, whereas in all other countries *C. coli* were also detected. In the southern EU MSs the presence of *C. coli* was more abundant and in some MSs more than half of the isolates belonged to this species. The reasons for the observed variation are not known but it may be hypothesised that climatic conditions, environmental reservoirs, broiler housing and age of slaughter that vary significantly form northern to southern Europe could partly explain these differences.

Unfortunately, the speciation results of the present baseline survey were not as robust as hoped for, as reported in the Part A report. Speciation of isolates using PCR-based methods detecting DNA sequences unique for the respective species, are more reliable to speciate isolates. Their use was not stipulated in the study protocol due to likely unavailability in some MSs and absence of harmonised protocols. However, results from countries, such as Austria, Belgium, the Czech Republic, France, Luxembourg, the Netherlands, Norway, Poland and Slovakia using PCR-based methods, confirms the variability of *Campylobacter* spp. frequency distribution across EU countries. A high proportion of *C. coli* in poultry meat were also reported from some other parts of the world (Meeyam et al., 2004; Padungtod et al., 2005; van Nierop et al., 2005).

6.5. Comparison of the notification rates of campylobacteriosis in humans and the *Campylobacter* prevalence in broiler batches and on broiler carcasses across the EU

Generally poultry meat, and especially broiler meat, is regarded as an important source for human *Campylobacter* infections. Recently an EFSA opinion confirmed that handling, preparation and consumption of broiler meat may be considered as an important source for human campylobacteriosis (EFSA, 2010b). Data from the present baseline survey show no association between the prevalence of *Campylobacter*-colonised broiler batches or *Campylobacter*-contaminated carcasses and the notification rates of human *Campylobacter* infections in different EU countries. However, this finding should be interpreted with extreme caution. The human campylobacteriosis notification rates varied highly among MSs and non-MSs. Considerable variation exists in the reporting systems for human illness, which might contribute to these differences (ECDC, 2008). In particular the ECDC report suggested that in some MSs, *Campylobacter* cases in humans are substantially under-reported. On the other hand, imported broiler meat which was not considered in the baseline survey, may be a source of human infections. Also the infections might be acquired through travel, non-domestic food, or non-food-borne routes (EFSA, 2009b).

The *Campylobacter*-speciation data from the human cases were considered too fragile to analyse in-depth in this report.

CONCLUSIONS

This Part B report provides results from further analyses of the baseline survey on *Campylobacter* in broiler batches and *Campylobacter* on broiler carcasses at slaughterhouse level. These are results regarding the association of 10 batch- and/or slaughterhouse level factors, on which data were collected via a questionnaire, and *Campylobacter* colonisation of batches and/or contamination of carcasses. In addition, further analyses of the identified *Campylobacter* species distribution across the EU, as well as the results of the investigation of the diagnostic sensitivities of the detection and enumeration methods used to estimate the prevalence of *Campylobacter*-contaminated broiler carcasses, are also included.

- In the survey, a strong positive association was observed at EU level between the likelihood of *Campylobacter* contamination of broiler carcasses with the presence of a *Campylobacter*-colonised batch. A *Campylobacter*-colonised broiler batch was about 30 times more likely to have the sampled carcass contaminated with *Campylobacter*, compared to a non-colonised batch. Thus, contaminated carcasses could also derive from non-colonised broiler batches, suggesting a potential for cross-contamination in the slaughterhouse environment. The *Campylobacter*-colonised broiler batches were also strongly associated with higher *Campylobacter* counts on the carcasses. These findings serve to emphasise the primary importance of on-farm control measures to manage the risks related to *Campylobacter* contamination of broiler carcasses.
- The risks for contamination of carcasses with *Campylobacter* and for higher *Campylobacter* counts on carcasses varied significantly between countries and between slaughterhouses within countries, even when other associated factors, such as the prevalence *Campylobacter*-colonised batches, were accounted for. These findings indicate that certain slaughterhouses are more capable than others in preventing *Campylobacter* contamination and in controlling the contamination and/or the *Campylobacter* counts on the carcasses. This implies that slaughterhouse processing offers an opportunity for *Campylobacter* risk mitigation.
- At EU level, the risk of *Campylobacter* contamination of carcasses or *Campylobacter* colonisation of batches increased with the increasing age of the broilers in the slaughter batch. Furthermore, the likelihood of having a *Campylobacter*-contaminated broiler carcass or a *Campylobacter*-colonised batch was higher in the second, third and fourth quarters of the year compared to the first one (January-March). The highest risk for contamination/colonisation was in the third quarter of the year (July to September). However, this possible seasonal effect should be verified in further studies in individual MSs.
- At the EU level, carcasses were also more likely to be contaminated with *Campylobacter* when processed later during the day, meaning with increasing time (hour) of sampling. However, the number of *Campylobacter* on these carcasses did not significantly increase during the day.
- Batches of broilers originating from previously thinned flocks were at higher risk of *Campylobacter* colonisation compared to non-thinned flocks. Furthermore, the risk of *Campylobacter* colonisation of batches varied significantly between countries and between slaughterhouses within countries, even when other associated factors were adjusted for.
- Delaying the time between sampling and testing increased the risk of detecting *Campylobacter* from the broiler batches or on the carcass samples.
- Additional analyses performed for countries with high or low prevalence of *Campylobacter*-colonised broiler batches, respectively, using the median[17] of prevalence (54.6%) as the cut-off point, disclosed no different outcomes compared to EU level analyses. However, in the case of countries with high or low prevalence of *Campylobacter*-contaminated carcasses, respectively, using the median of prevalence (62.5%) as the cut-off point, there was variation between significantly associated factors. For the higher prevalence country group, the flock production

[17] Two non-MSs, Norway and Switzerland, were included to calculate the EU median prevalence.

type and chilling methods were additionally found to be associated with carcass contamination, with carcasses originating from organic flocks and carcasses chilled by mixed methods being at higher risk of *Campylobacter* contamination.

- The analyses of the *Campylobacter* contamination of carcasses and of the results of *Campylobacter* colonisation of batches showed that important proportions, ranging overall from 18% to 50%, of unexplained variance was attributable to slaughterhouse-specific factors for which no data were gathered during the survey. The magnitude of such an unexplained variance was bigger for the group of countries with prevalence above the EU median compared to the group of countries with prevalence below the EU median.
- Investigation of the culture method results used to estimate the prevalence of *Campylobacter*-contaminated broiler carcasses showed that the diagnostic sensitivity of the detection test may have varied between MSs. This implied that the actual true prevalence of *Campylobacter*-contaminated carcasses might be more importantly underestimated for some MSs. This possible detection test (misclassification) bias also warrants caution while interpreting the results of the EU level analyses of factors associated with the prevalence of *Campylobacter*-contaminated broiler carcasses or with the prevalence of *Campylobacter*-colonised broiler batches.

RECOMMENDATIONS

- MSs are invited to consider the factors found to be associated, at EU level, with *Campylobacter*-contaminated broiler carcasses and/or with *Campylobacter*-colonised broiler batches when designing national *Campylobacter* control programmes for broiler meat or broiler flocks.
- An integrated control programme that addresses both the primary production and the slaughter process would seem to be important in strategies to prevent or reduce subsequent contamination of the broiler carcass and to improve protection of public health.
- Further national studies to identify more closely, at batch- and slaughterhouse-level, the factors that put broiler batches and carcasses at risk of becoming colonised or contaminated with *Campylobacter* in a country are recommended.
- The standardisation of the time between sampling and testing as well as of the quality control of laboratory testing methods should be considered of importance by MSs when designing national *Campylobacter* control programmes. In particular, it is recommended that MSs investigate further the sensitivity of the *Campylobacter* detection method.

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LIST OF APPENDICES

A. STATISTICAL METHODOLOGY USED IN THE ANALYSIS OF THE REPORT B PRODUCED FOR THE EU-WIDE BASELINE SURVEY ON THE PREVALENCE OF *CAMPYLOBACTER* IN BROILER BATCHES AND ON BROILER CARCASSES

1. Data import and management

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

The data provided by EFSA contain information at the level of the samples within a slaughterhouse. For simplification purposes, several new dichotomous variables were created, e.g., "vcc_spp" stands for the results of *Campylobacter* spp. in the carcass sample and combines the results of detection and enumeration tests.

2. Methodology and tools for descriptive analysis

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

In what follows, some detailed discussion is provided on the tests used to study association between *Campylobacter* prevalence and the independent variables. Note that this association is studied using the data at the Community-level, that is including European MSs and two non-MSs.

Chi-square tests

Consider two categorical variables *X* and *Y*, *X* having *I* levels and *Y* having *J* levels. The *IJ* possible combinations of outcomes can be displayed in a contingency table having *I* rows for the categories of *X*, and *J* columns for the categories of *Y*. For instance, Table 13 represents the notation used in such a 2 X 4 table.

Table 13. **Notation for the cross classification of *Campylobacter* spp. prevalence in broiler carcasses by quarter of sampling**

***Campylobacter* spp.**	**Quarter of sampling**				**Total**
	Jan-Mar 2008	**Apr-Jun 2008**	**Jul-Sep 2008**	**Oct-Dec 2008**	
No	n_{11}	n_{12}	n_{13}	n_{14}	n_{1+}
Yes	n_{21}	n_{22}	n_{23}	n_{24}	n_{2+}
Total	n_{+1}	n_{+2}	n_{+3}	n_{+4}	n

The null hypothesis H_0 of independence is equivalent to cell probabilities satisfying $\{\pi_{ij} = \pi_{i+}\pi_{+j}\}$. For a sample of size *n* with cell counts $\{n_{ij}\}$, the values $\{\mu_{ij} = n\pi_{ij}\}$ represent the expected frequencies, i.e. the values of the expectations $\{E(n_{ij})\}$ when H_0 is true. The sample cell counts can then be compared to the expected frequencies to judge whether the data contradict H_0. If the null

hypothesis is true, then n_{ij} should be close to μ_{ij} in each cell. The larger the differences $\{n_{ij} - \mu_{ij}\}$, the stronger the evidence against H_0. In practice, μ_{ij} can be estimated by $\hat{\mu}_{ij} = n_{i+}n_{+j}/n$.

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

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

which is asymptotically distributed according to a Chi-square with $(I-1)(J-1)$ degrees of freedom. It is difficult to evaluate whether the available sample size is large enough for these asymptotic results to be valid. A general rule of thumbs is given by $\{\mu_{ij} \geq 5\}$. When the sample size is small, one can resort to inferences using *exact* distributions rather than large-sample approximations (Agresti, 2002).

Spearman correlation

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

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

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

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

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

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

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

3. Methodology and tools for the regression analysis

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

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

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

1) the assumptions on the binomial distribution are violated; and

2) the sample is not drawn at random (but essentially stratified).

Indeed:

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

Clustering

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

- *Ignore the correlation.* While this typically leaves the consistency of point estimation intact, the same is not true for measures of precision. In the case of a "positive" correlation (i.e. samples within a slaughterhouse are more alike than between slaughterhouses), then ignoring this aspect of the data, just as ignoring overdispersion, overestimates precision and hence underestimates standard errors and widths of CIs.
- *Account for correlation*. The existence of correlation is recognised but considered as a nuisance characteristic. A crude way of correcting for clustering is done by computing a so-called *design effect*. Roughly, the design effect is a factor comparing the precision under simple random sampling with the precision of the actual design. Standard errors, computed as if the design had been simple random sampling, can then be artificially inflated using the design effect.
- *Model correlation*. In contrast to the previous view-point, one can have a genuine scientific interest in the correlation itself. The intra-class correlation should be addressed in order to obtain valid statistical inferences, and specialised methods which model the correlation should be used.

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

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

There are two routes to introduce randomness into the model parameters. The first approach introduces the random effects on the probability scale, such as the beta-binomial model (Skellam, 1948). The second approach introduces the random effects in the linear predictor, yielding the classical mixed-effects models (Stiratelli et al., 1984). A random-effects logistic regression model is

an example of the second approach, where it is assumed that the number of positive samples y_{ij} in slaughterhouse *j* in country *i* follow a binomial distribution:

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

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

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

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

In addition to the slaughterhouse-specific random intercepts, the possible risk factors are taken account of, which are given by categorical, ordinal and continuous variables. No global intercept is used; the slaughterhouse effect is explained by the random intercept and the covariates show the possible influences on the *Campylobacter* result.

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

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

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

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

Weighting

Most statistical procedures analyse the data as if they were collected as a simple random sample. As a result, these procedures may lead to biased estimates and may underestimate the variability present in the data, when the data actually arise from complex surveys. Assigning weights to the observations is one possible approach to correct for the differences between the complex survey design and simple random sampling. In general, by using weights, the 'total population' is tentatively reconstructed, in order to avoid that certain strata or subpopulations are over- or underrepresented. Below the weighting scheme for broiler carcasses samples and for pooled caeca samples is described.

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

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

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

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

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

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

Multicollinearity analysis among risk factors

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

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

Note that the VIF is equal to 1 when $R^2_k = 0$, i.e., when X_k is not linearly related to the other risk factors. When $R^2_k \neq 0$, then the VIF will be higher than 1, indicating an inflated variance for the estimated regression coefficients due to correlations among the risk factors.

A maximum VIF exceeding 10 is frequently interpreted as an indication of multicollinearity.

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

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

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

Intra-cluster Correlation Coefficient

This is a measure to describe the similarity of the responses on the outcome within a slaughterhouse (cluster). The Intra-cluster Correlation Coefficient (ICC) ranges between 0 and 1. For a random intercept model, the ICC considers the variance of the random intercepts and the variance of the standard logistic density (Molenberghs and Verbeke, 2005). Let *z* be a matrix of estimable functions and *D* be the unstructured variance-covariance matrix of the random effects b_i. Thus, the ICC is given by the following formula:

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

Negative Binomial Regression Model

Usually when the objective of the study is to assess the relationship between a set of explanatory variables and a response variable, which is the result of a counting process, one uses a Poisson regression. Based on this model, the mean and the variance are assumed to be exactly the same; however the data do not necessarily exhibit such behaviour.

A more flexible model makes use of the Negative Binomial distribution, where the variance is a function of the mean and also includes a overdispersion parameter. (Agresti, 2002). The probability density function for a negative binomial distribution is as follows:

$$\Pr(Y = y) = \frac{\Gamma(y+\tau)}{\Gamma(y+1)\Gamma(\tau)}\left(1+\frac{\lambda}{\tau}\right)^{-\tau}\left(1+\frac{\tau}{\lambda}\right)^{-y}, y = 0,1,2, \ldots,$$

where $\Gamma(.)$ denotes the gamma function, τ is the overdispersion parameter and the mean and the variance of the distribution are given respectively by:

$E(Y) = \lambda$, $Var(Y) = \lambda + \tau\lambda^2$

This model is taken into account to describe the distribution of the positive counts of the *Campylobacter* enumeration results.

B. DESCRIPTIVE ANALYSIS OF POTENTIAL FACTORS ASSOCIATED WITH *CAMPYLOBACTER*-COLONISED BROILER BATCHES

In this appendix a descriptive analysis of the potential factors associated with *Campylobacter*-colonised broiler batches is provided.

Flock production type

Table 14. **Number and percentage of broiler batches by flock production type in the EU*, 2008**

Flock production type	Total	
	N	%
Conventional	9,092	*91.7*
Free-range standard	593	*6.0*
Free-range organic	101	*1.0*
Unknown	130	*1.3*
Total	9,916	*100.0*

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

Most of the samples originate from flocks where the production type is conventional (Table 14). For this group the prevalence of *Campylobacter* spp. (Figure 11) seems lowest.

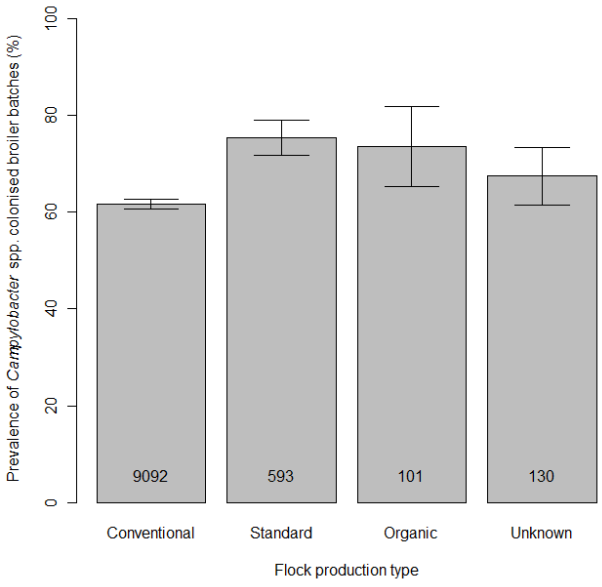

Figure 11. **Prevalence of *Campylobacter*-colonised broiler batches by flock production type in the EU*, 2008**

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

Table 15. **Pearson Chi-square test for independence between the flock production type and *Campylobacter*-colonisation result in the broiler batch**

	Chi-square statistic (*P*-value)
Campylobacter spp.	50.2 (<0.0001)

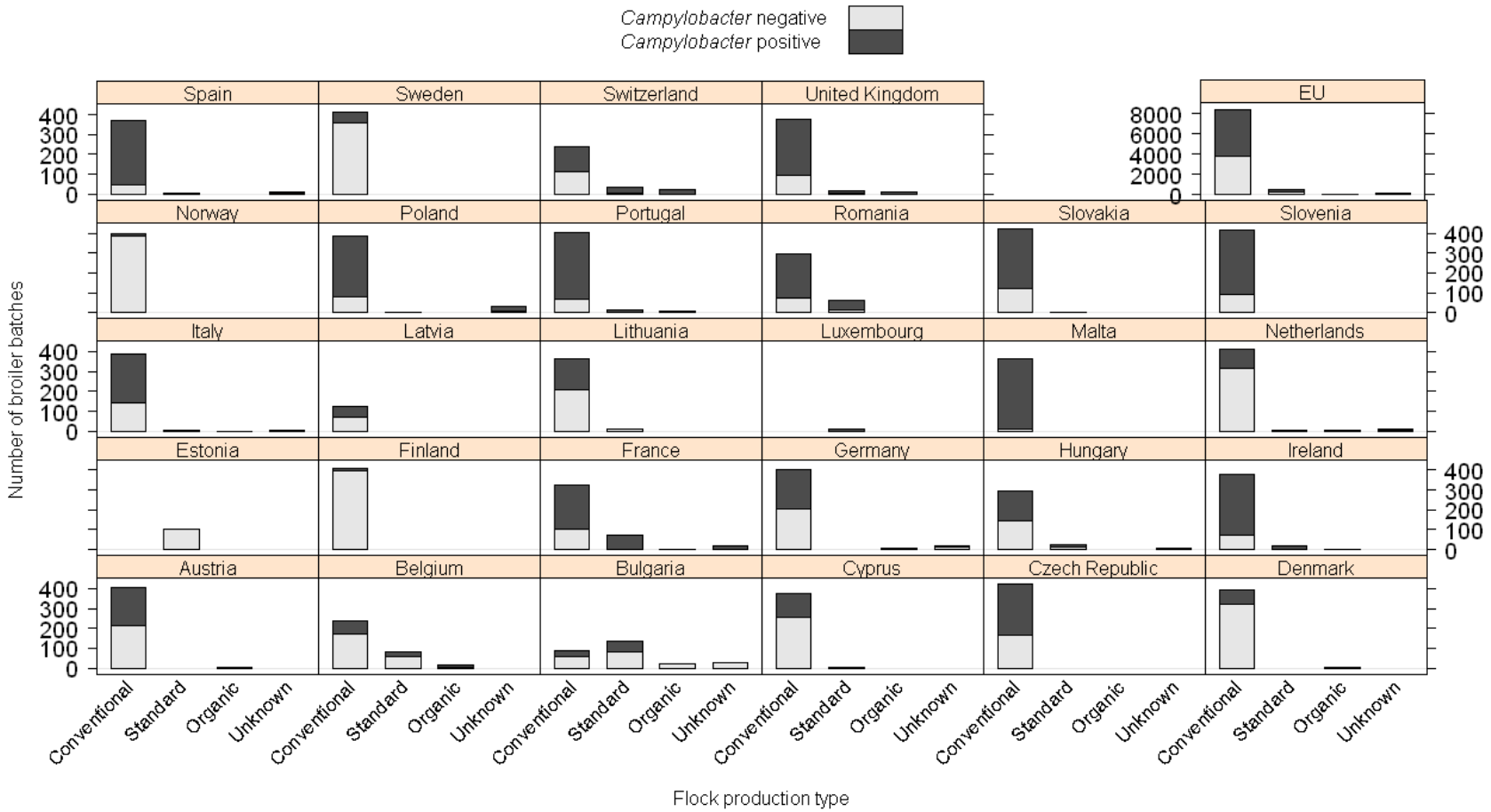

Figure 12. **Multiple bar graphs of estimated (weighted) frequency counts of *Campylobacter*-positive and -negative broiler batches by flock production type, by country and in the EU*, 2008**

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

Previous thinning in the flock

Table 16. **Number and percentage of broiler batches by previous thinning in the flock in the EU*, 2008**

Previous thinning in the flock	Total	
	N	**%**
No	5,721	*57.7*
Yes	2,717	*27.4*
Unknown	1,478	*14.9*
Total	**9,916**	***100.0***

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

Table 17. **Pearson Chi-square test for independence between the previous thinning in the flock and *Campylobacter*-colonisation result in the broiler batch**

	Chi-square statistic (*P*-value)
Campylobacter spp.	133.5 (<0.0001)

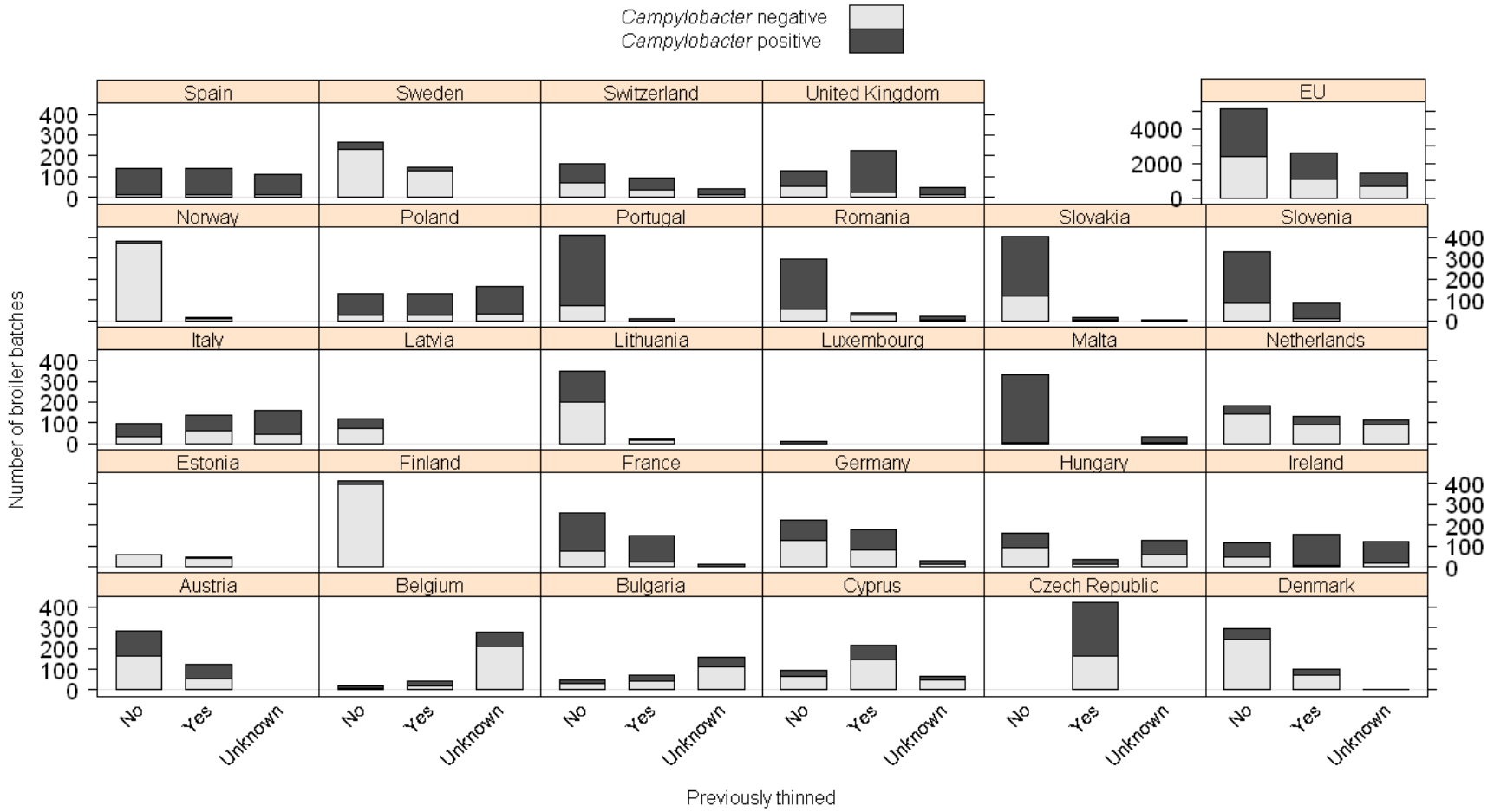

Figure 13. **Multiple bar graphs of estimated (weighted) frequency counts of *Campylobacter*-positive and -negative broiler batches, by previous thinning in the flocks of origin, by country and in the EU*, 2008**

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

Age of broilers

Table 18. **Summary statistics (minimum, maximum, mean and standard deviation) for the age of the broilers (in days) for the *Campylobacter*-colonisation result in broiler batches in the EU*, 2008**

Age of broilers				Total
Min	**Max**	**Mean**	**Std**	**N**
20	150	41.4	8.7	9916

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

Based on the linear trend test there is a linear association between the age of broilers and *Campylobacter*-colonisation result in the broiler batches (Table 19).

Table 19. **Cochran-Mantel-Haenszel Chi-square test for the linear trend between the age of broilers and *Campylobacter*-colonisation result in the broiler batch**

	Linear trend statistics (*P*-value)
Campylobacter spp.	608.7 (<0.0001)

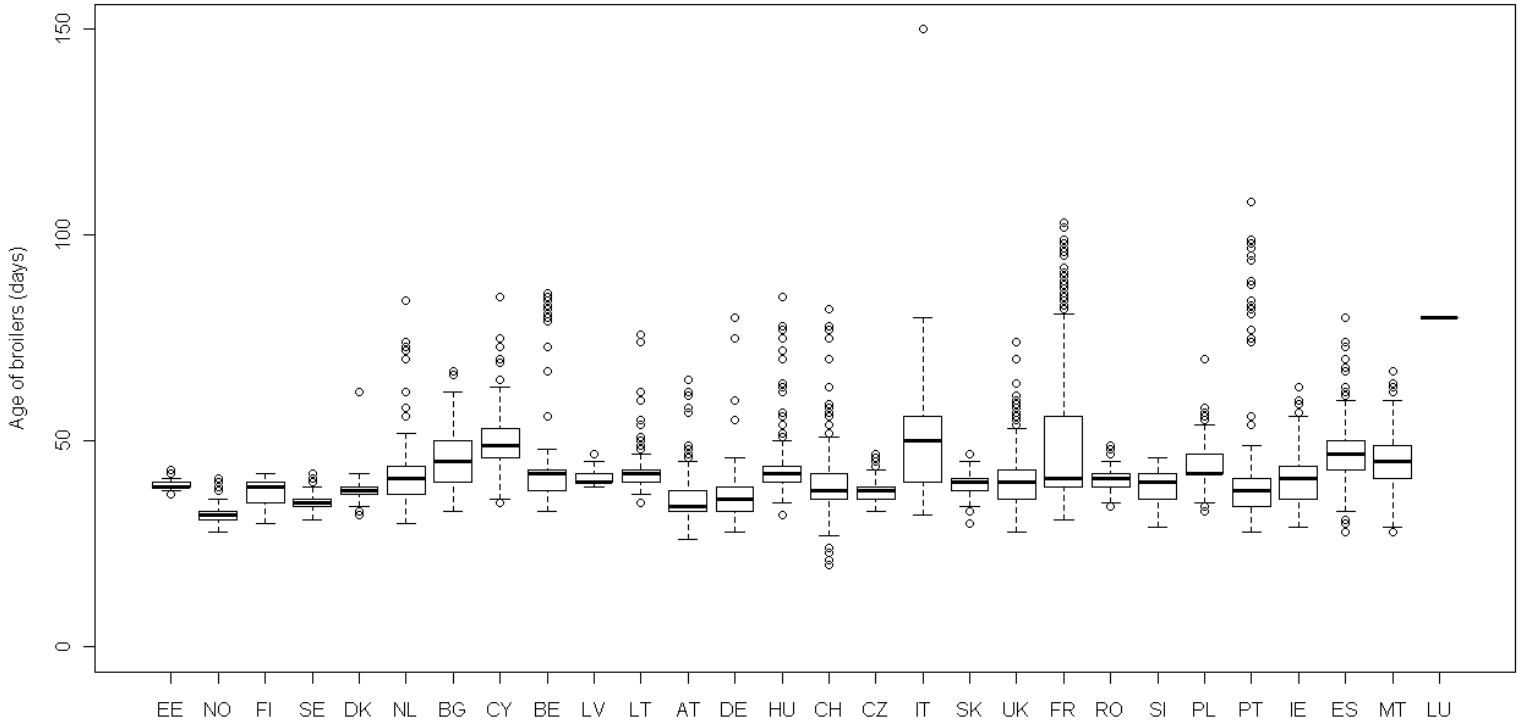

Figure 14. **Boxplot of the age of broilers by country ranked according to the prevalence of *Campylobacter*-colonised broiler batches, 2008***

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

In the boxplots, the bottom section of the box represents the first quartile of the distribution and the top section of the box the third quartile, 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).

Quarter of sampling

Table 20 shows the number of samples according to the quarter of sampling and Table 21 presents the Pearson Chi-square test to assess the independence between quarter of sampling and prevalence.

Table 20. **Number and percentage of broiler batches by quarter of sampling in the EU*, 2008**

Quarter of sampling	Total	
	N	%
1	1,998	*20.2*
2	2,584	*26.1*
3	2,586	*26.1*
4	2,748	*27.7*
Total	**9,916**	***100.0***

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

Table 21. **Pearson Chi-square test for independence between the quarter of sampling and *Campylobacter*-colonisation result in the broiler batch**

	Chi-square statistic (*P*-value)
Campylobacter spp.	236.5 (<0.0001)

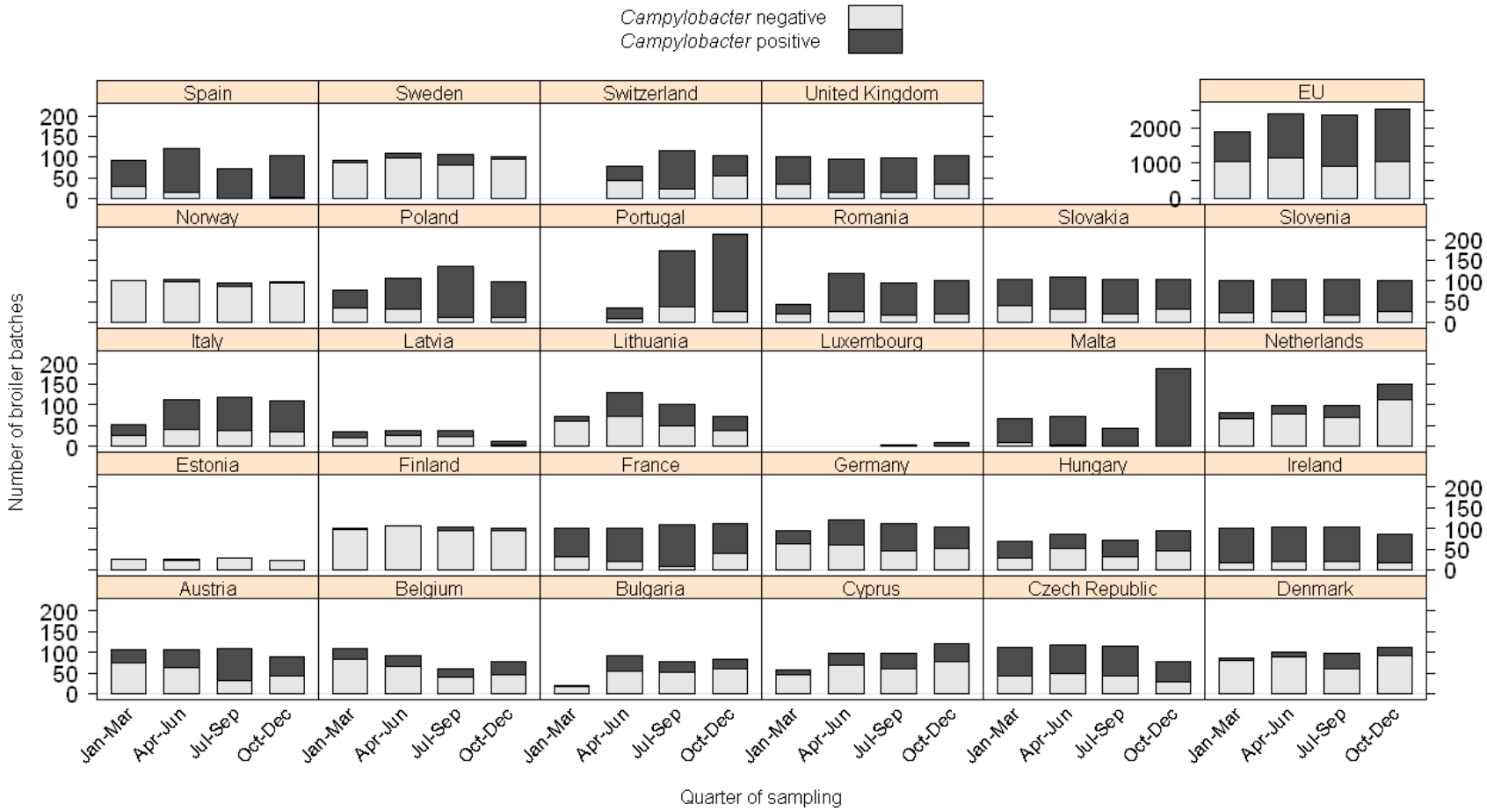

Figure 15. **Distribution of the *Campylobacter*-colonised broiler batches by quarter of sampling, by country and in the EU*, 2008**

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

Time (hour) of sampling

Table 22. **Number and percentage of broiler batches by time (hour) of sampling during the day in the EU*, 2008**

Time (hour) of sampling during the day	Total	
	N	%
<9 am	4,412	*44.5*
9 - <12am	3,335	*33.6*
12am – <3pm	1,187	*12.0*
≥3pm	982	*9.9*
Total	9,916	*100.0*

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

Clearly most of the samples were collected during the morning (78.1%), before 12 am, whereas less than 10% were collected after 3 pm (Table 22).

Taking into account the ordinal nature of the variable a Cochran Mantel-Haenszel Chi-square test can be used to assess the association between hour of sampling during the day and the *Campylobacter*-colonisation result in the broiler batch (Table 23). This test considers the ordinal character of the variables involved and it is also applied whenever the risk factor is ordinal.

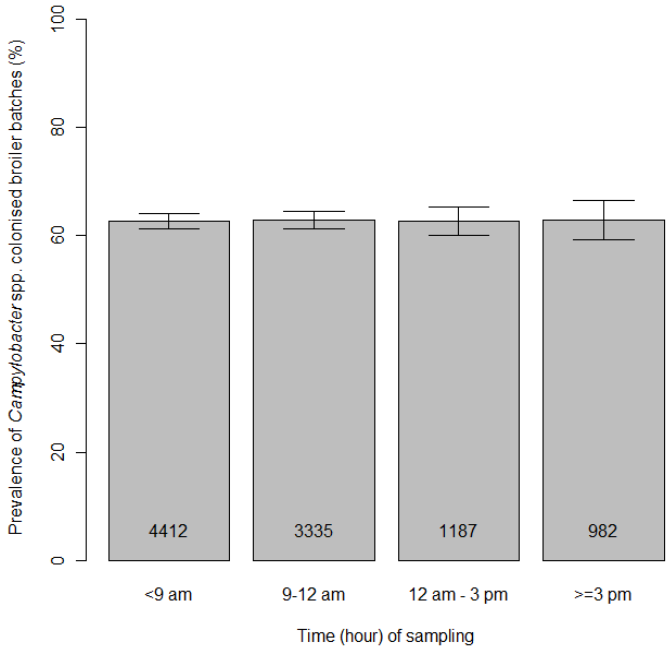

Figure 16. **Prevalence of *Campylobacter*-colonised broiler batches by time (hour) of sampling during the day in the EU*, 2008**

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

Table 23. **Cochran-Mantel-Haenszel Chi-square test for the linear trend between the time (hour) of sampling during the day and *Campylobacter*-colonisation result in the broiler batch**

	Linear trend statistics (*P*-value)
Campylobacter spp.	0.0089 (0.9248)

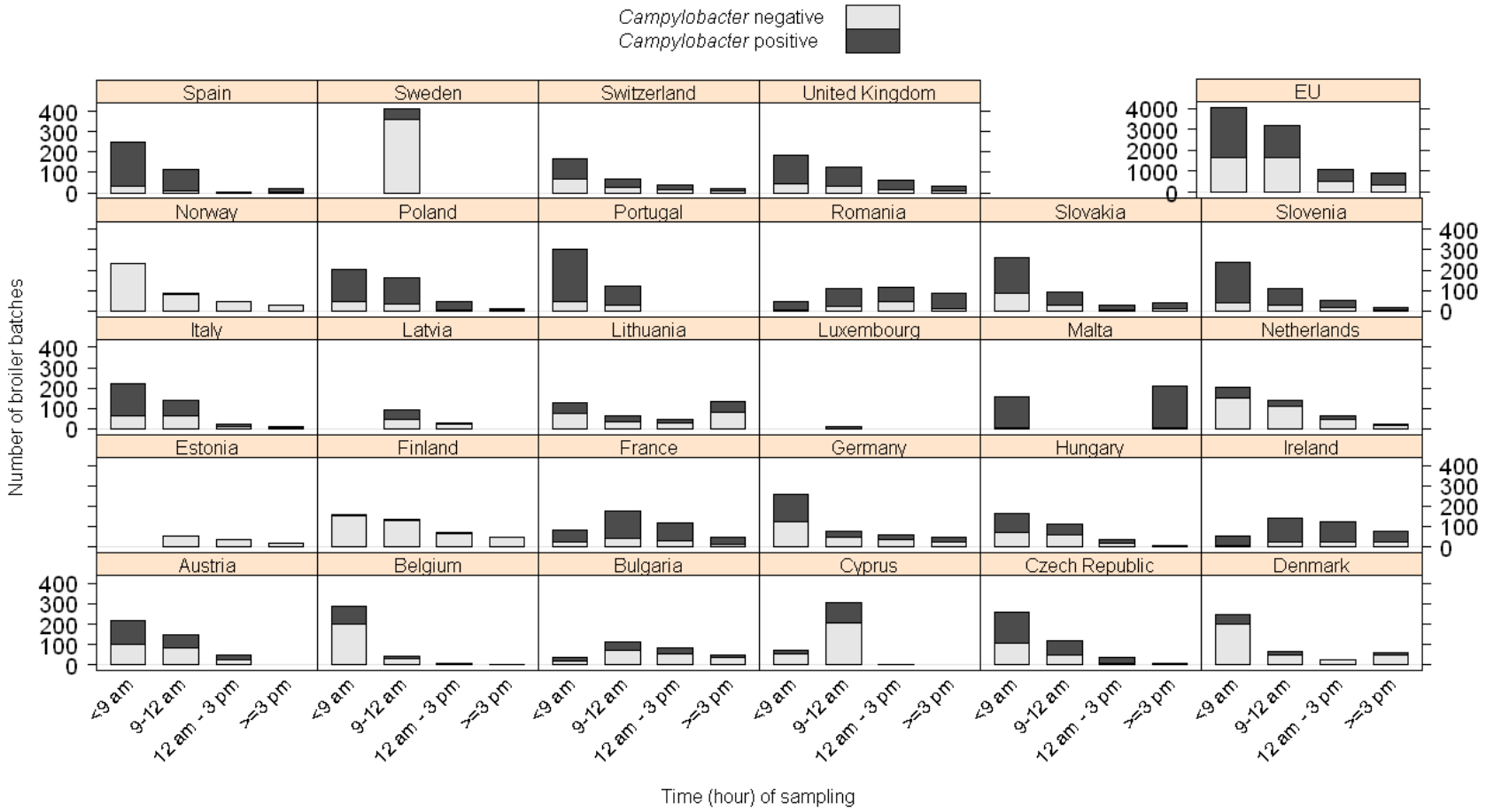

Figure 17. **Distribution of the *Campylobacter*-colonised broiler batches by time (hour) of sampling, by country and in the EU*, 2008**

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

Hours between sampling and testing

Table 24. **Number and percentage of broiler batches by hours between sampling and testing in the EU*, 2008**

Hours between sampling and testing	Total	
	N	%
<24 hours	3,094	*31.2*
24 hours - <36 hours	4,329	*43.7*
36 hours - <48 hours	1,098	*11.1*
48 hours - <60 hours	862	*8.7*
60 hours - <72 hours	240	*2.4*
72 hours - <80 hours	293	*3.0*
Total	9,916	*100.0*

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

According to Table 24, most of the caeca samples were analysed for *Campylobacter* within 36 hours (74.9% of the samples). From Figure 18, the probability of having a positive result is bigger in the samples that were analysed between 24 and 36 hours, compared to the probability of having a positive result in the samples that were analysed within 24 hours.

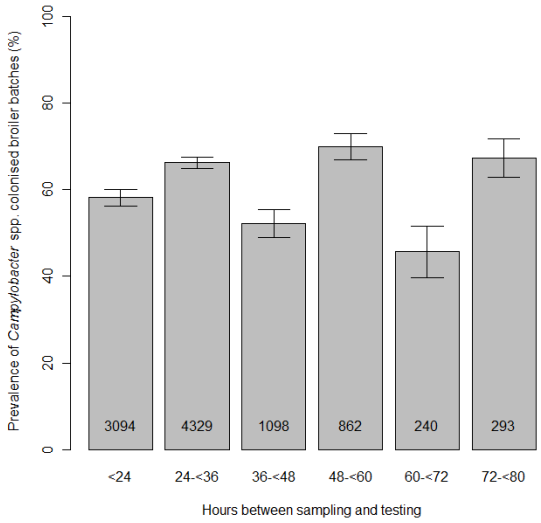

Figure 18. **Prevalence of *Campylobacter*-colonised broiler batches by hours between sampling and testing in the EU*, 2008**

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

Based on the linear trend it can be concluded that there is association between the hours between sampling and testing and the result of *Campylobacter* in the broiler batches (Table 25).

Table 25. **Cochran-Mantel-Haenszel Chi-square test for the linear trend between the hours between testing and sampling and *Campylobacter*-colonisation result in the broiler batch**

	Linear trend statistics (*P*-value)
Campylobacter spp.	4.8 (0.0290)

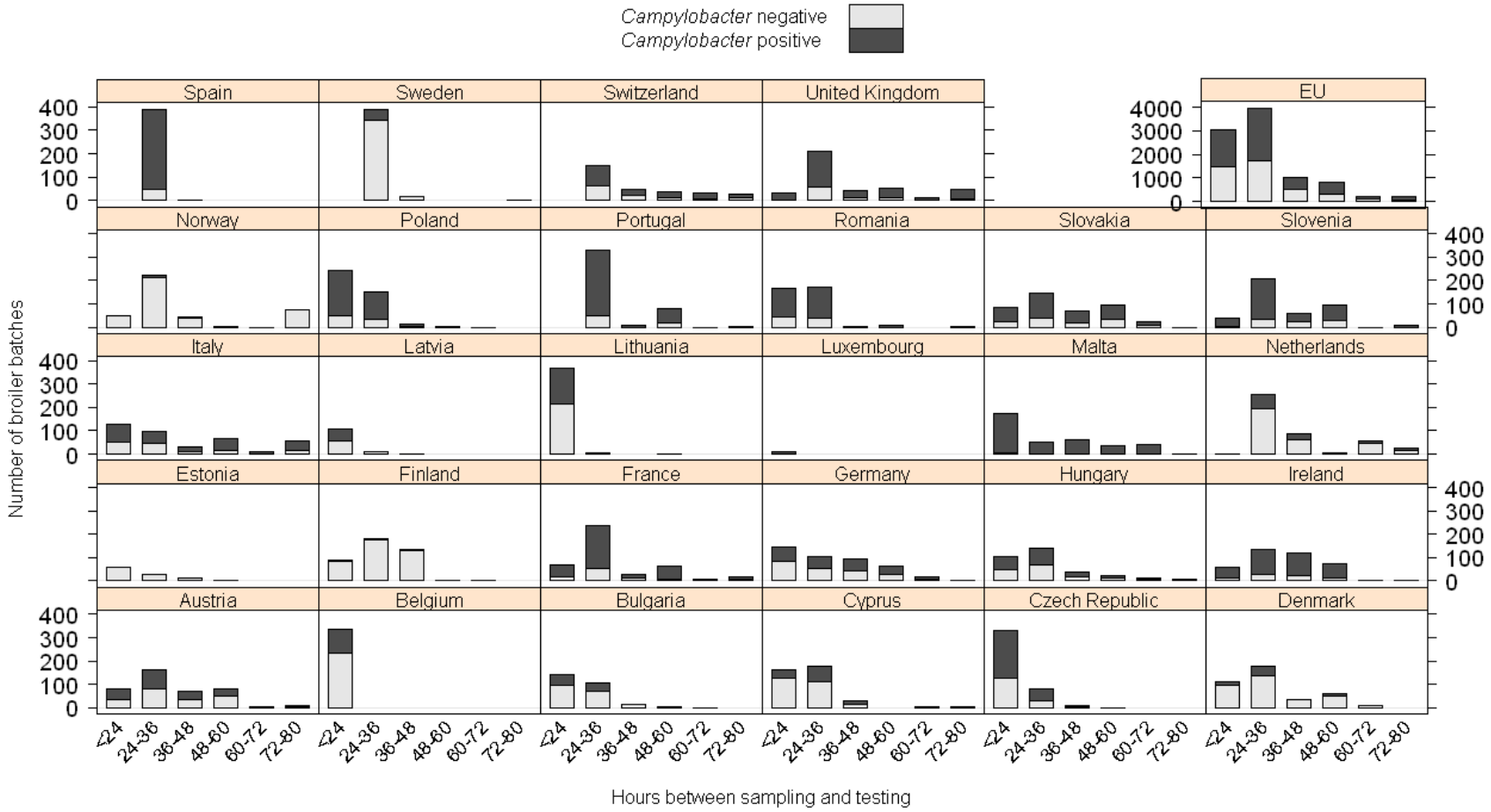

Figure 19. **Distribution of the *Campylobacter*-colonised broiler batches by hours between sampling and testing, by country and in the EU*, 2008**

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

C. FINAL MODEL FOR *CAMPYLOBACTER*-COLONISATION RESULT IN THE BROILER BATCH: VARIANCE INFLATION FACTOR VALUES AND VARIANCE OF RANDOM INTERCEPTS

Table 26. **Variance Inflation Factor values for factors potentially related to *Campylobacter*-colonised broiler batches**

Risk Factor	VIF
Flock production type	1.18
Previous thinning in the flock	1.10
Age of broilers	1.28
Quarter of sampling	1.01
Time (hour) of sampling	1.15
Hours between sampling and testing	1.16

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

The variance of the random effects (effect of slaughterhouses) in the final regression model was significantly different from zero. The Wald test statistic was 23.7 and the *P*-value (<0.001) was calculated using a 50:50 mixture of Chi-square distributions with 0 and 1 degrees of freedom, respectively, given that the value under the null hypothesis lies on the border of the parameter space (Molenberghs and Verbeke, 2005).

Table 27. **Final logistic mixed-effects model for factors associated with *Campylobacter*-colonised broiler batches: variance of slaughterhouse-specific random intercepts, in the EU, 2008**

	Estimate	Standard Error	*P*-value
Variance of the random intercepts	1.12	0.23	<0.001

D. FULL MODELS FOR CAMPYLOBACTER-COLONISED BROILER BATCHES IN COUNTRIES WITH PREVALENCE ABOVE THE EU MEDIAN PREVALENCE AND COUNTRIES WITH PREVALENCE BELOW THE EU MEDIAN PREVALENCE[18]

Table 28. **Comparison of the full models for *Campylobacter* result in broiler batches between countries with prevalence below the EU median prevalence and above the EU median prevalence– Odds ratio and 95% confidence intervals for the risk factors and *P*-value of the type III test for fixed effects**

	Variable	Levels	OR	Lower	Upper	*P*-value
Countries with prevalence below the EU* median prevalence	Flock production type	*Unknown*	0.65	0.31	1.36	0.5737
	Reference category: *Conventional*	*Organic*	1.04	0.29	3.73	
		Standard	1.21	0.74	1.96	
	Previous thinning in the flock	*Unknown*	1.01	0.68	1.51	0.0064
	Reference category: *No*	*Yes*	1.49	1.13	1.95	
	Age of broilers (scale 10 days)		1.58	1.21	2.05	0.0007
	Quarter of sampling	*IV*	1.78	1.31	2.43	<0.0001
	Reference category: *I*	*III*	2.54	1.79	3.63	
		II	1.49	1.05	2.13	
	Time (hour) of sampling during the		0.98	0.95	1.02	0.4210
Countries with prevalence above the EU* median prevalence	Flock production type	*Unknown*	1.11	0.41	3.05	0.3890
	Reference category: *Conventional*	*Organic*	1.46	0.38	5.65	
		Standard	2.71	0.86	8.56	
	Previous thinning in the flock	*Unknown*	1.03	0.66	1.62	0.0002
	Reference category: *No*	*Yes*	2.12	1.46	3.08	
	Age of broilers (scale 10 days)		2.08	1.56	2.77	<0.0001
	Quarter of sampling	*IV*	2.45	1.65	3.63	<0.0001
	Reference category: *I*	*III*	5.65	3.67	8.70	
		II	2.56	1.83	3.57	
	Time (hour) of sampling during the day		1.04	1.01	1.08	0.0691

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

[18] Two non-MSs, Norway and Switzerland, were included to calculate the EU median prevalence.

E. FINAL MODEL FOR *CAMPYLOBACTER COLI*-COLONISED BROILER BATCHES, VARIANCE OF RANDOM INTERCEPTS

Table 29. **Final model [(a)] for *Campylobacter coli*-colonised broiler batches: odds ratio estimates and corresponding 95% confidence intervals and *P*-value of the type III test for the risk factors***

Factor	Level	Odds ratio	95% CI		*P*-value
Age of broilers (scale 10 days)		1.33	1.18	1.50	<0.0001
Quarter of sampling	*IV*	1.80	1.38	2.35	<0.0001
Reference category: *I*	*III*	2.96	2.19	4.01	
	II	1.44	1.06	1.95	

(a): Estimates and standard errors were assessed using a mixed-effects model with the effect of slaughterhouses included as a random effect (random intercepts) and with the factor 'country' included as a fixed effect.

* All ORs were adjusted for the country and the factor 'hours between sampling and testing' was only used to adjust the model.

Table 30. **Final logistic mixed-effects model for factors associated with *Campylobacter coli*-colonised broiler batches: variance of slaughterhouse-specific random intercepts, in the EU, 2008.**

	Estimate	Standard Error
Variance of the random intercepts	0.85	0.15

F. DESCRIPTIVE ANALYSIS OF POTENTIAL FACTORS ASSOCIATED WITH *CAMPYLOBACTER*-CONTAMINATED BROILER CARCASSES PREVALENCE

In this appendix a descriptive analysis of the potential factors associated with *Campylobacter*-colonised broiler batches prevalence is provided.

Flock production type

Table 31. **Number and percentage of broiler carcasses by flock production type in the EU*, 2008**

Flock production type	Total	
	N	%
Conventional	9,154	*91.4*
Free-range organic	114	*1.1*
Free-range standard	619	*6.2*
Unknown	130	*1.3*
Total	**10,017**	***100.0***

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

The prevalences of *Campylobacter* based on broiler carcasses are very similar in the free-range organic, free-range standard and in the unknown categories of the flock production type and much lower for the conventional category (Figure 20).

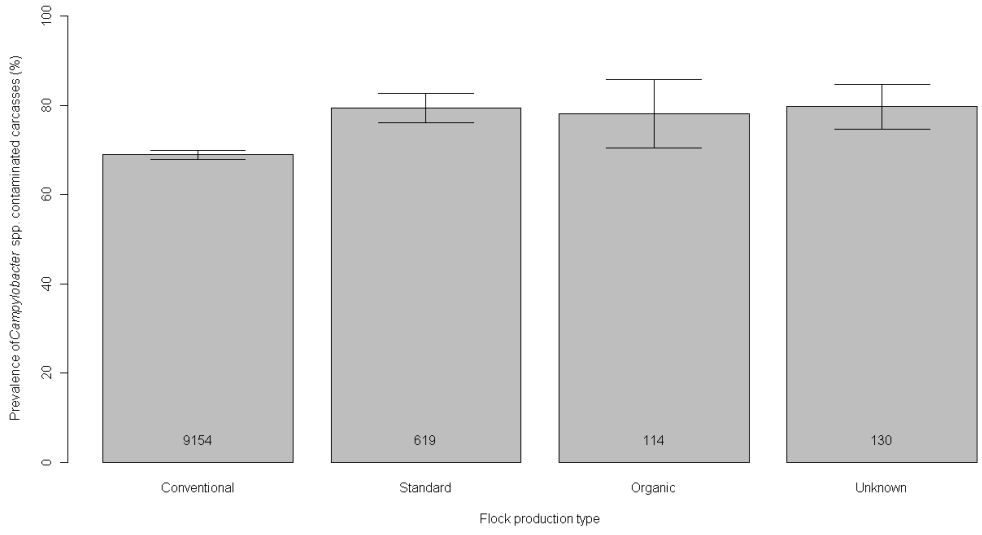

Figure 20. **Prevalence of *Campylobacter*-contaminated broiler carcasses by flock production type in the EU*, 2008**

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

Table 32. **Pearson Chi-square test for the independence between flock production type and *Campylobacter* result on the broiler carcass**

	Chi-square statistic (*P*-value)
Campylobacter spp.	43.03 (<0.0001)

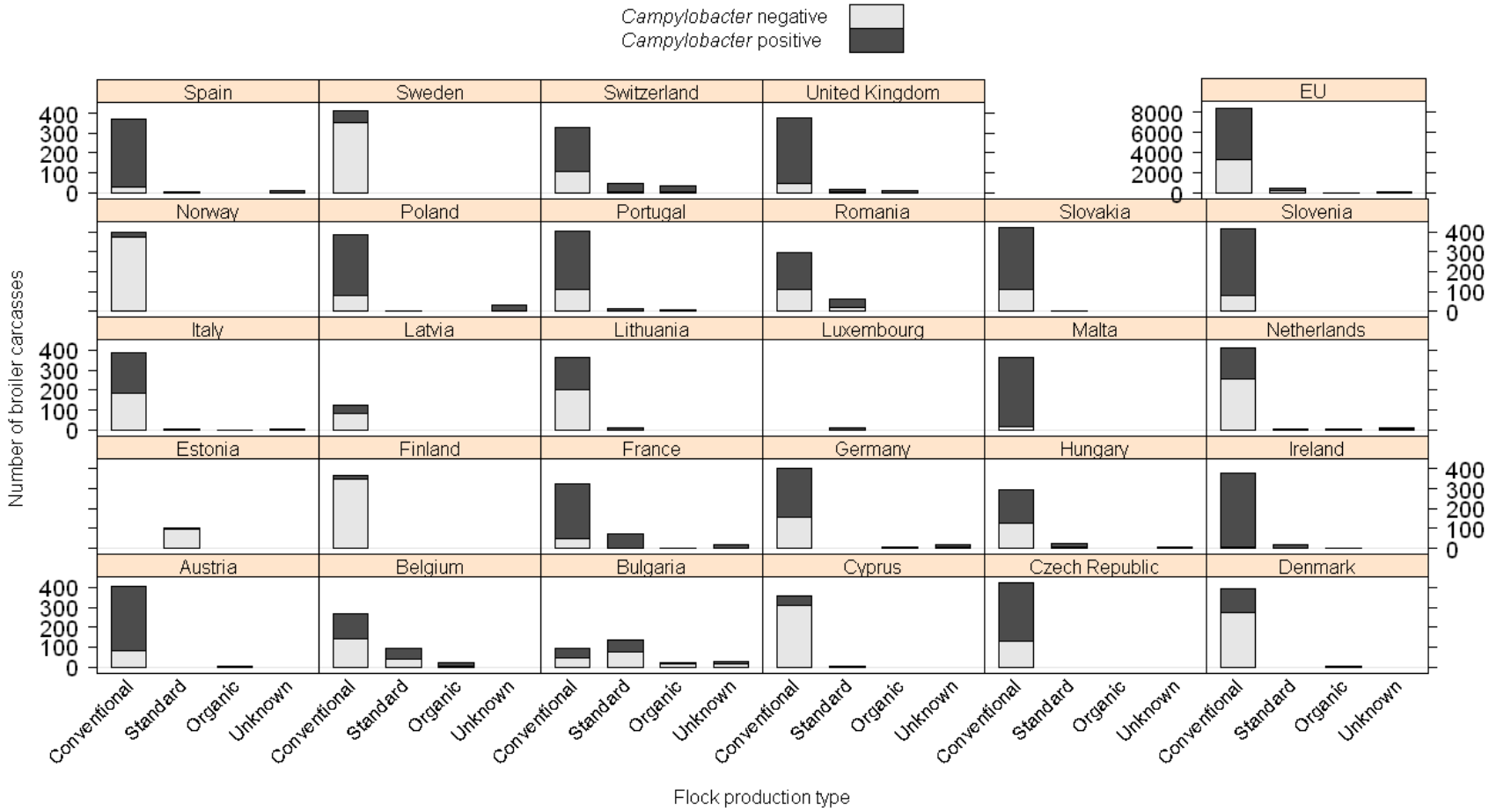

Figure 21. **Multiple bar graphs of estimated (weighted) frequency counts of *Campylobacter*-positive and -negative broiler carcasses by flock production type, by country and in the EU*, 2008**

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

Previous thinning in the flock

Table 33. **Number and percentage of broiler carcasses by previous thinning in the flocks of origin in the EU*, 2008**

Previous thinning in the flock	Total	
	N	%
No	5,723	*57.1*
Yes	2,760	*27.6*
Unknown	1,534	*15.3*
Total	**10,017**	***100.0***

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

Similar to the results obtained in the broiler batches, the prevalence of *Campylobacter*-contaminated broiler carcasses is lower when there has been no previous thinning in the flock (Figure 22).

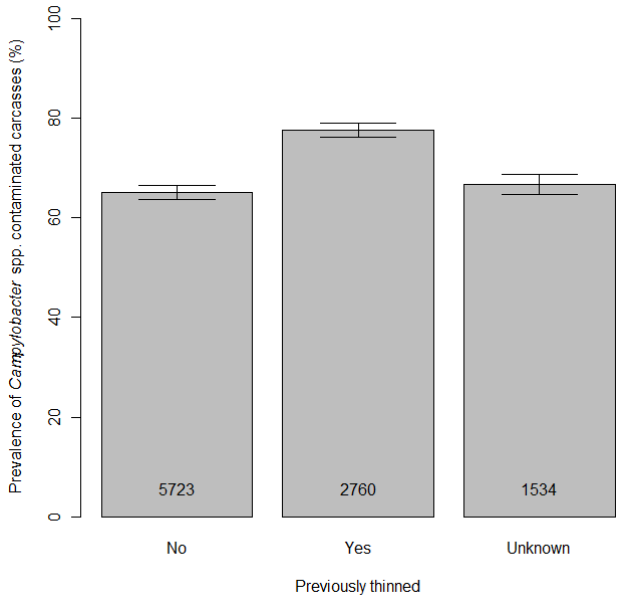

Figure 22. **Prevalence of *Campylobacter*-contaminated broiler carcasses by previous thinning in the flock in the EU*, 2008**

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

Table 34. **Pearson Chi-square test for independence between the previous thinning in the flock and *Campylobacter* result on the broiler carcass**

	Chi-square statistic (*P*-value)
Campylobacter spp.	156.6 (<0.0001)

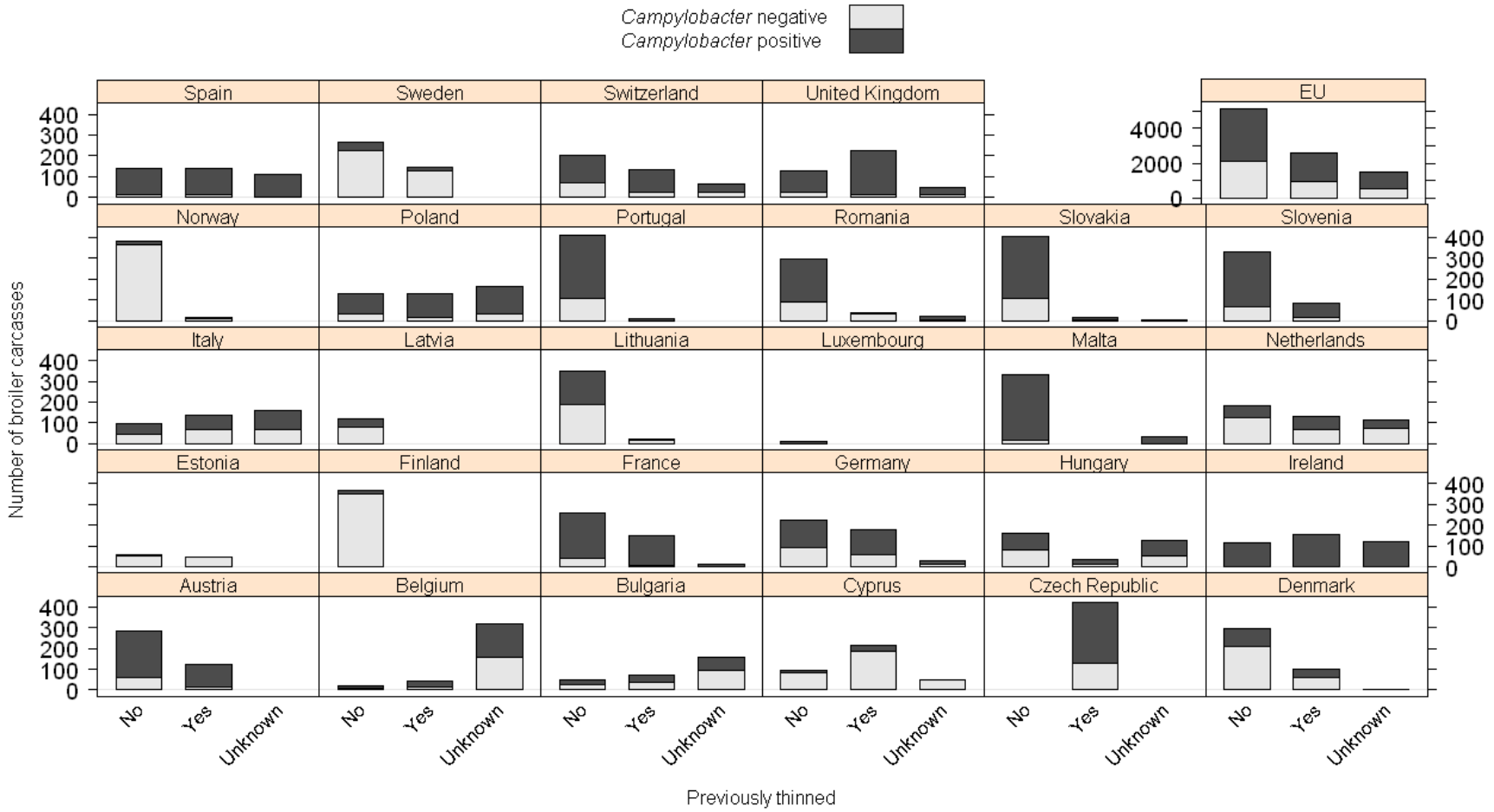

Figure 23. **Multiple bar graphs of estimated (weighted) frequency counts of *Campylobacter*-positive and-negative broiler carcasses by previous thinning in the flock, by country and in the EU*, 2008**

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

Age of broilers

Table 35. **Summary statistics (minimum, maximum, mean and standard deviation) for the age of broilers (in days) for the *Campylobacter* result on the broiler carcass in the EU*, 2008**

Age of broilers				Total
Min	**Max**	**Mean**	**Std**	**N**
17	150	41.5	8.8	10,017

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

Based on the linear trend test, there is an association between this outcome and the age of broilers (Table 36).

Table 36. **Cochran-Mantel-Haenszel Chi-square test for the linear trend between the age of broiler and *Campylobacter* result on the broiler carcass**

	Linear trend statistics (*P*-value)
Campylobacter spp.	326.06 (<0.0001)

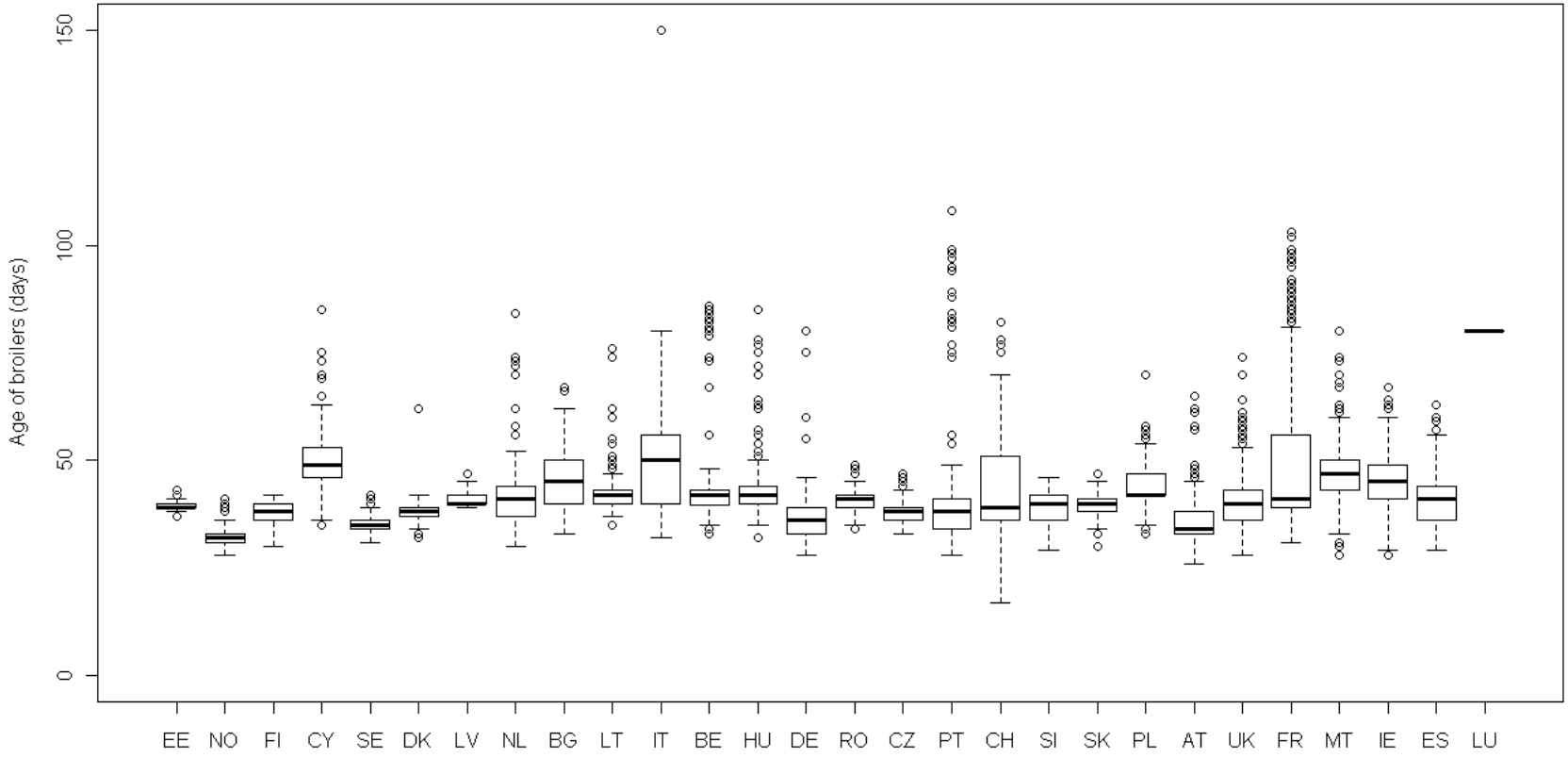

Figure 24. **Boxplot of the age of broilers, by country ranked according to the prevalence of *Campylobacter*-contaminated carcasses*, 2008**

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

In the boxplots, the bottom section of the box represents the first quartile of the distribution and the top section of the box the third quartile, 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).

Quarter of sampling

Table 37. **Number and percentage of broiler carcasses by quarter of sampling in the EU*, 2008**

Quarter of sampling	Total	
	N	%
1	2,082	*20.8*
2	2,574	*25.7*
3	2,607	*26.0*
4	2,754	*27.5*
Total	10,017	*100.0*

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

Here also Pearson Chi-square shows evidence of the association between the risk factor and the outcome (Table 38).

Table 38. **Pearson Chi-square test for independence between the quarter of sampling and *Campylobacter* result on the broiler carcass**

	Chi-square statistic (*P*-value)
Campylobacter spp.	78.2 (<0.0001)

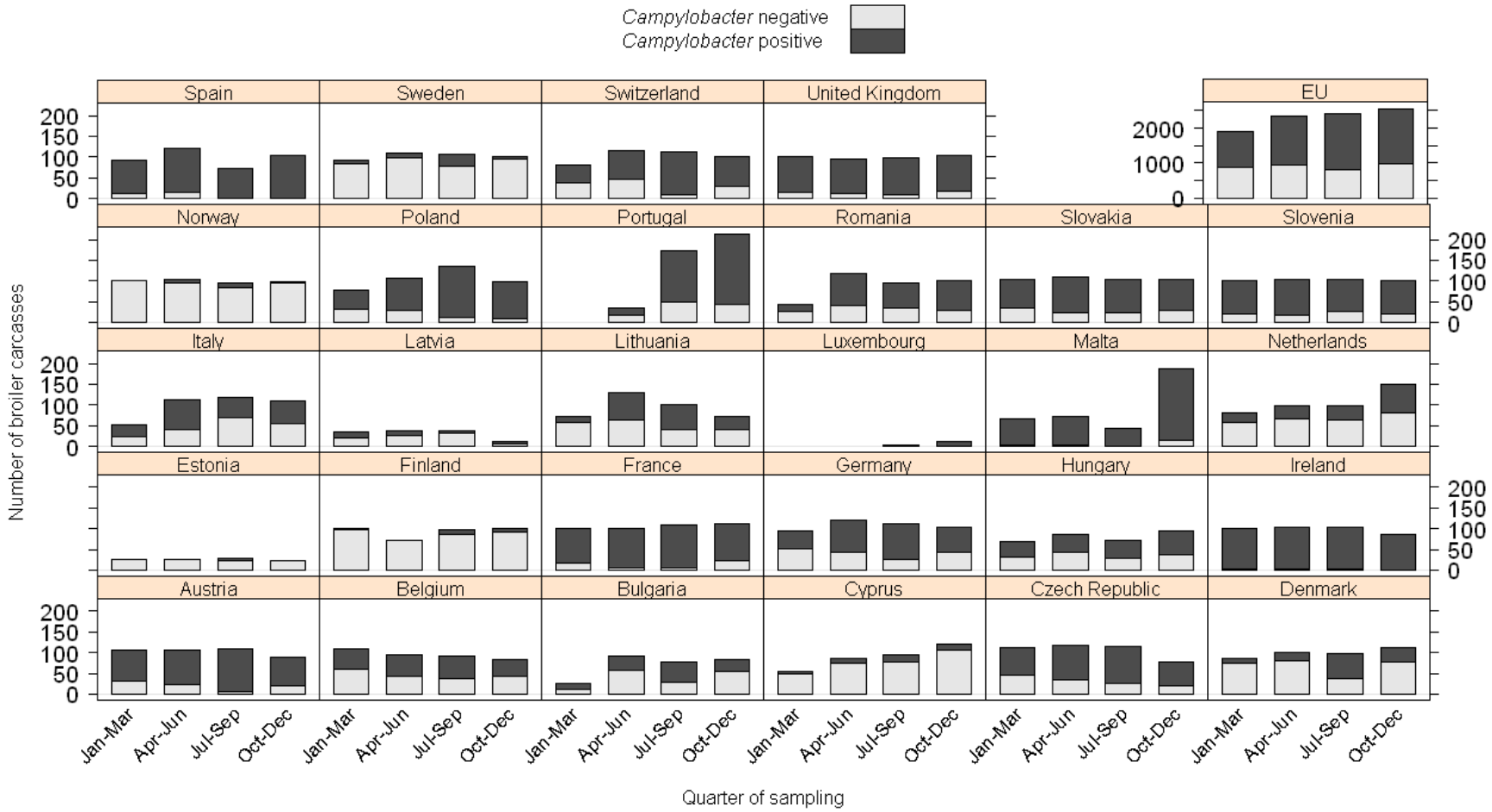

Figure 25. **Multiple bar graphs of estimated (weighted) frequency counts of *Campylobacter*-positive and -negative broiler carcasses by quarter of sampling, by country and in the EU[*], 2008**

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

Time (hour) of sampling

Table 39. **Number and percentage of broiler carcasses by time (hour) of sampling during the day in the EU*, 2008**

Time (hour) of sampling	Total	
	N	%
<9 am	4,517	*45.1*
9 - <12am	3,335	*33.3*
12am – <3pm	1,184	*11.8*
≥3pm	981	*9.8*
Total	**10,017**	***100.0***

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

Table 40. **Cochran-Mantel-Haenszel Chi-square test for the linear trend between the time (hour) of sampling during the day and *Campylobacter* result on the broiler carcass**

	Linear trend statistics (*P*-value)
Campylobacter spp.	13.32 (0.0003)

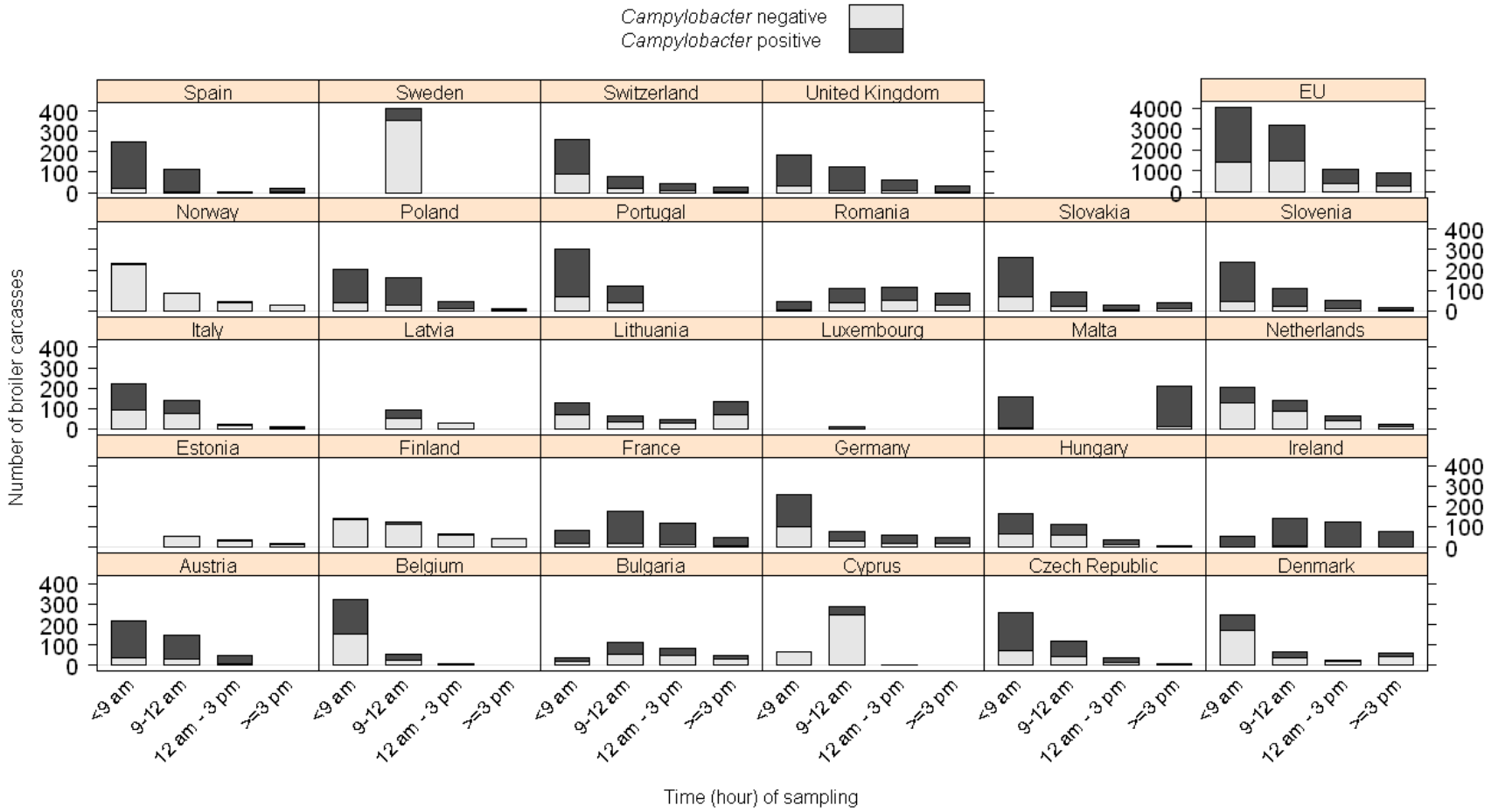

Figure 26. **Multiple bar graphs of estimated (weighted) frequency counts of *Campylobacter*-positive and -negative broiler carcasses by time (hour) of sampling, by country and in the EU*, 2008**

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

Hours between sampling and testing

Table 41. **Number and percentage of broiler carcasses by hours between sampling and testing in the EU*, 2008**

Hours between sampling and testing	Total	
	N	%
<24 hours	3,170	*31.7*
24 hours - < 36 hours	4,141	*41.3*
36 hours - < 48 hours	1,141	*11.4*
48 hours - < 60 hours	991	*9.9*
60 hours - < 72 hours	259	*2.6*
72 hours - < 80 hours	315	*3.1*
Total	**10,017**	***100.0***

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

Similar to the results that were observed in the broiler batches, the prevalence of *Campylobacter*-contaminated broiler carcasses is bigger for the samples that were analysed between 24 and 36 hours after the collection, compared to the prevalence of those which were analysed within 24 hours (Figure 27).

Table 42. **Cochran-Mantel-Haenszel Chi-square test for the linear trend for the hours between sampling and testing and *Campylobacter* result on the broiler carcass**

	Linear trend statistics (*P*-value)
Campylobacter spp.	4.08 (0.043)

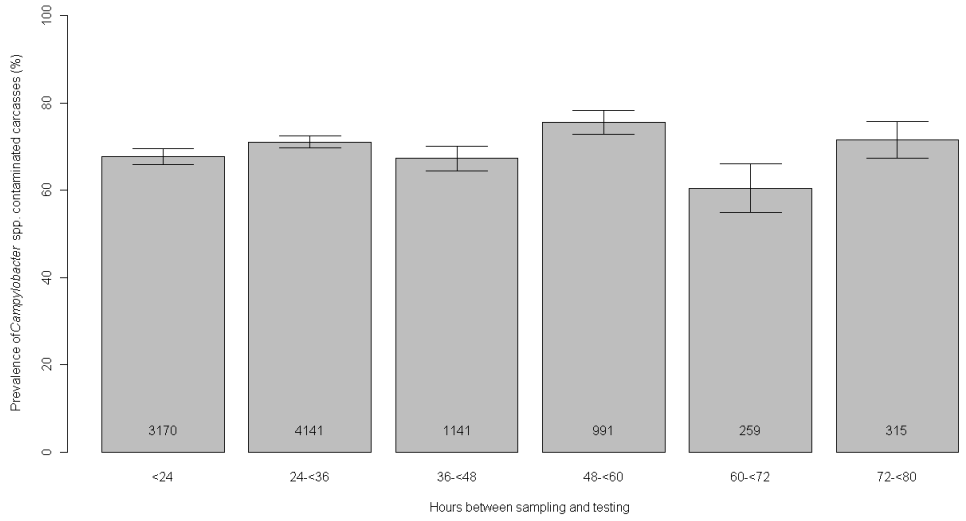

Figure 27. **Prevalence of *Campylobacter*-contaminated broiler carcasses by hours between sampling and testing in the EU*, 2008**

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

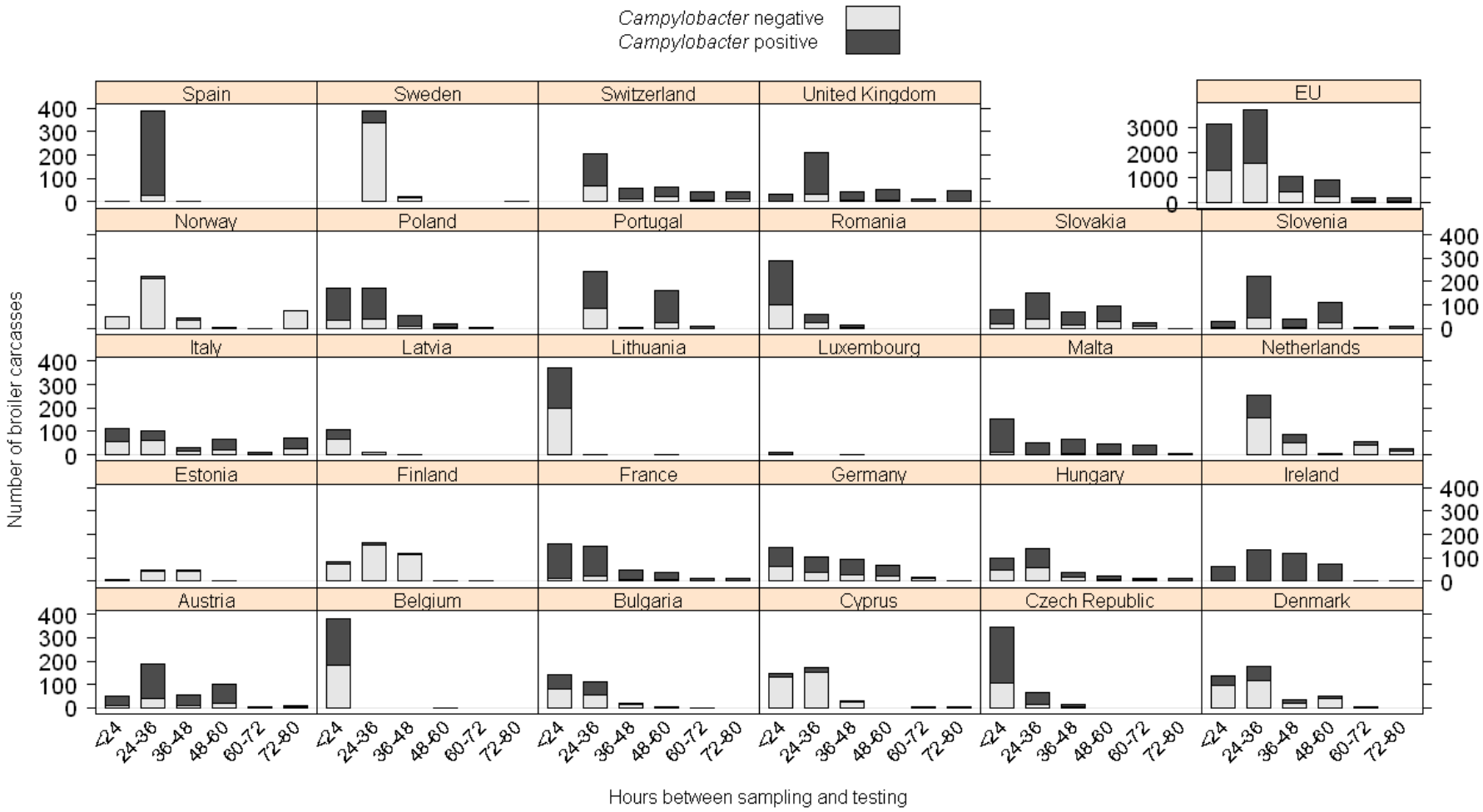

Figure 28. **Distribution of the *Campylobacter*-contaminated carcasses by hours between sampling and testing, by country and in the EU*, 2008**

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

Capacity of the slaughterhouses

Table 43. **Number and percentage of broiler carcasses by capacity of slaughterhouse in the EU*, 2008**

Capacity of the slaughterhouse	Total	
	N	%
<100,000	184	*1.8*
100,000-499,999	338	*3.4*
500,000-999,999	340	*3.4*
1,000,000-4,999,999	1,261	*12.6*
5,000,000-9,999,999	1,887	*18.8*
>10,000,000	6,007	*60.0*
Total	**10,017**	***100.0***

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

Most of the samples come from slaughterhouses with capacity for more than a million broilers (Table 43). From Figure 29 and Table 44, no clear relationship between the *Campylobacter* result on the broiler carcass and the capacity of the slaughterhouses can be observed.

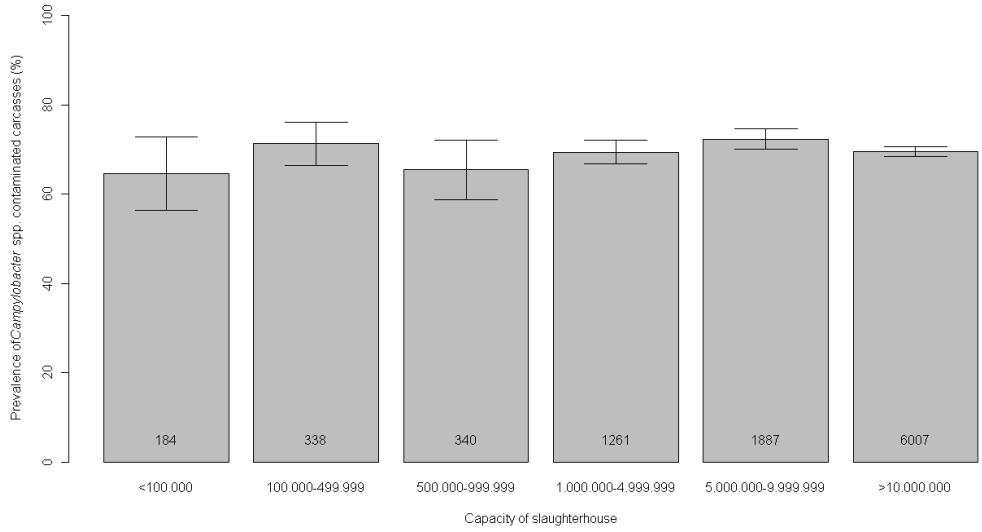

Figure 29. **Prevalence of *Campylobacter*-contaminated broiler carcasses by capacity of slaughterhouse in the EU*, 2008**

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

Table 44. **Cochran-Mantel-Haenszel Chi-square test for the linear trend between the capacity of slaughterhouse and *Campylobacter* result on the broiler carcass**

	Linear trend statistics (*P*-value)
Campylobacter spp.	0.21 (0.65)

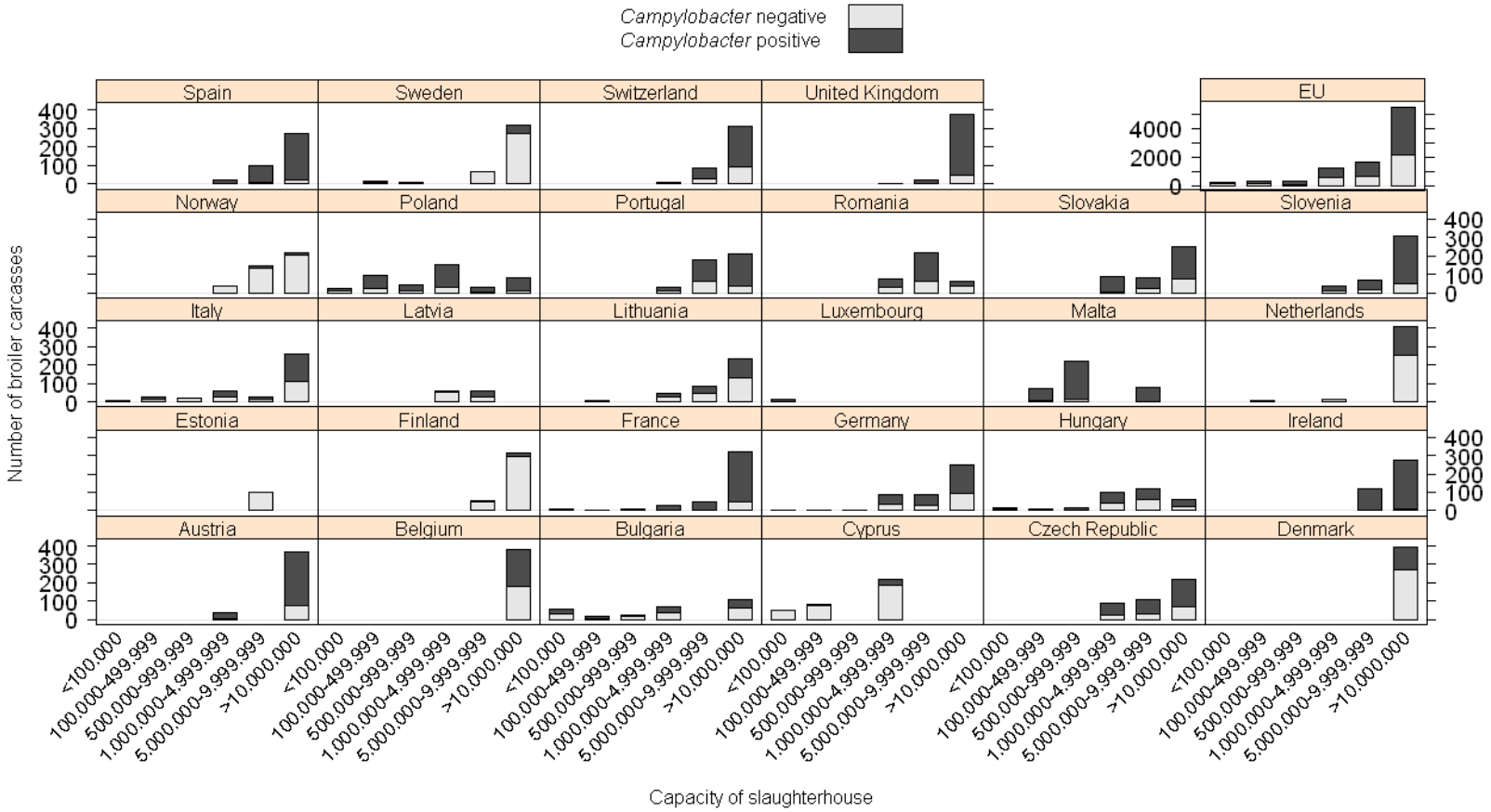

Figure 30. **Multiple bar graphs of estimated (weighted) frequency counts of *Campylobacter*-positive and -negative broiler carcasses by capacity of the slaughterhouse, by country and in the EU*, 2008**

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

Type of chilling

The 'mixed' category was created comprising all observed combinations of chilling methods, due to the small number of samples.

Table 45. **Number and percentage of broiler carcasses by type of carcass chilling in the EU*, 2008**

Type of chilling of carcass	Total	
	N	%
Air	7,222	*72.1*
Immersion	764	*7.6*
Spray	1,554	*15.5*
Mixed	477	*4.8*
Total	**10,017**	***100.0***

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

The lowest prevalence of *Campylobacter*-contaminated carcasses according to the type of carcass chilling is for the spray category, whereas the highest are in the categories immersion and mixed (Figure 31). Based on Table 45, the difference in prevalence is significant among the categories of type of carcass chilling.

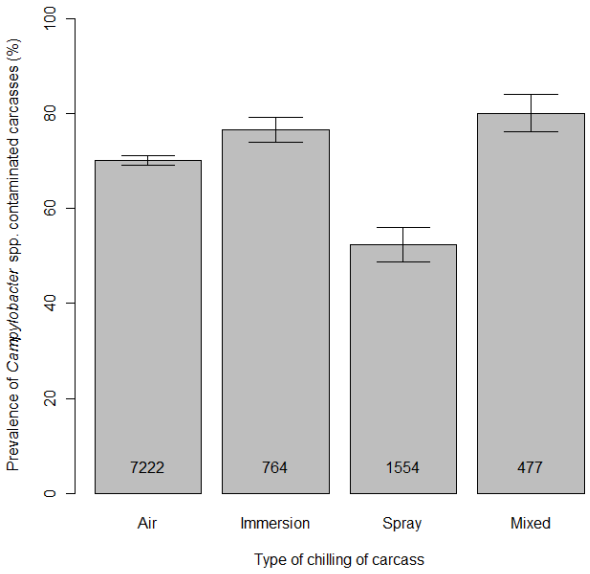

Figure 31. **Prevalence of *Campylobacter*-contaminated broiler carcasses by type of carcass chilling in the EU*, 2008**

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

Table 46. **Pearson Chi-square test for independence between type of carcass chilling and *Campylobacter* result on the broiler carcass**

	Chi-square statistic (*P*-value)
Campylobacter spp.	144.5 (<0.0001)

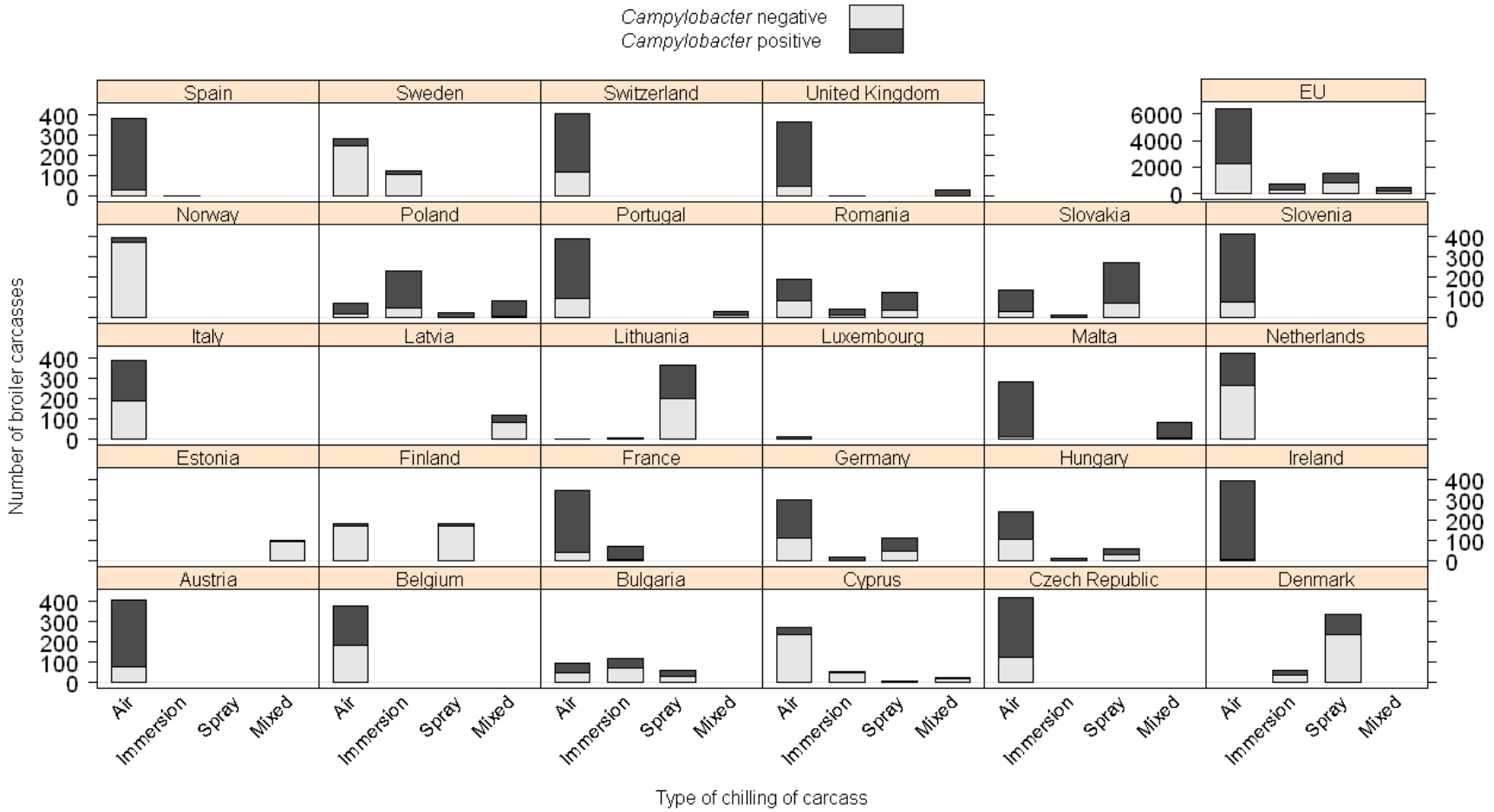

Figure 32. **Multiple bar graphs of estimated (weighted) frequency counts of *Campylobacter*-positive and -negative broiler carcasses by type of chilling, by country and in the EU*, 2008**

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

***Campylobacter*-colonisation result in the broiler batch**

Table 47. **Number and percentage of broiler carcasses by *Campylobacter*-colonisation result in the broiler batch in the EU*, 2008**

***Campylobacter*-colonisation result in the broiler batch**	**Total**	
	N	**%**
Negative	4,594	*45.9*
Positive	5,243	*52.3*
Unknown	180	*1.8*
Total	**10,017**	***100.0***

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

Clearly there is a strong association between the results of the test based on broiler batches and broiler carcasses (Table 48).

Table 48. **Pearson Chi-square test for independence between *Campylobacter*-colonisation result in the broiler batch and *Campylobacter* result on the broiler carcass**

	Chi-square statistic (*P*-value)
Campylobacter spp.	3,590.3 (<0.0001)

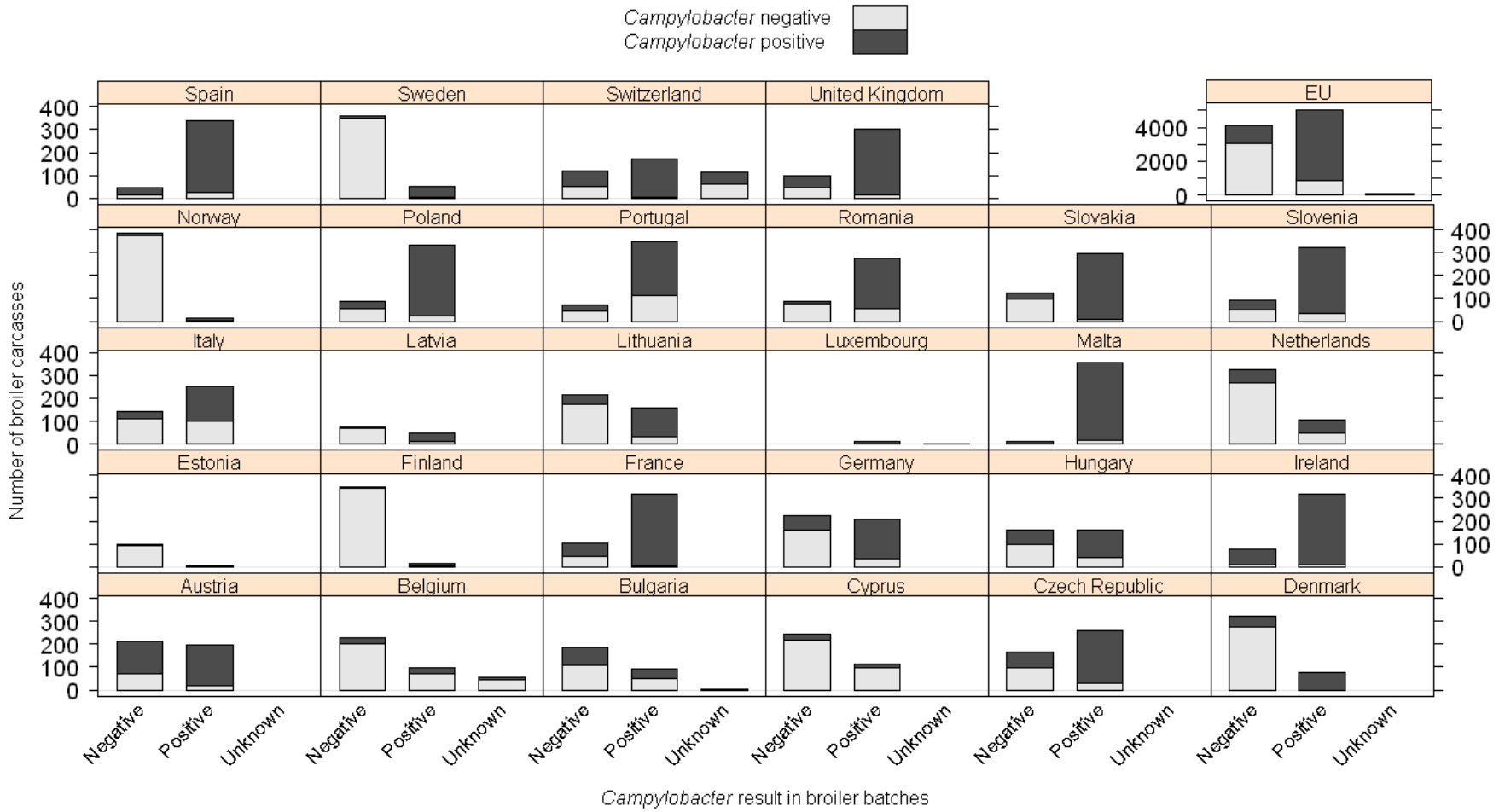

Figure 33. **Multiple bar graphs of estimated (weighted) frequency counts of *Campylobacter*-positive and -negative broiler carcasses by *Campylobacter*-colonisation result in the broiler batch, by country and in the EU*, 2008**

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

***Salmonella*-contamination result on the broiler carcass**

Table 49. **Number and percentage of broiler carcasses by *Salmonella*-contamination result on the broiler carcass in the EU*, 2008**

Salmonella-contamination result on the broiler carcass	Total	
	N	%
Negative	8,783	*87.7*
Positive	1,216	*12.1*
Unknown	18	*0.2*
Total	**10,017**	***100.0***

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

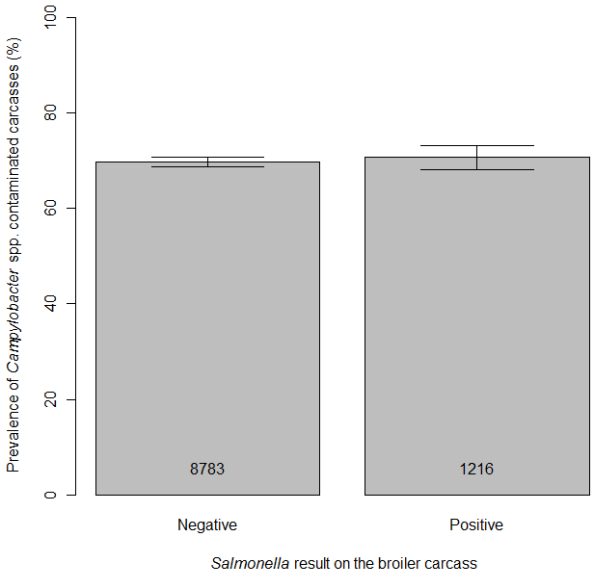

Figure 34. **Prevalence of *Campylobacter*-contaminated broiler carcasses by the *Salmonella*-contamination result on the broiler carcass[a] in the EU*, 2008**

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

(a): A total of 18 samples were excluded from this analysis, as the *Salmonella*-contamination result on broiler carcasses was missing.

There is no evidence of association between the result of *Campylobacter* and the result of *Salmonella* contamination based on broiler carcass samples. The prevalence of *Campylobacter* on the *Salmonella*-positive and -negative samples is nearly 70% (Figure 34).

Table 50. **Pearson Chi-square test for independence between result of *Salmonella* contamination on the broiler carcass and *Campylobacter*-contamination result on the broiler carcass**

	Chi-square statistic (*P*-value)
Campylobacter spp.	2.19 (0.34)

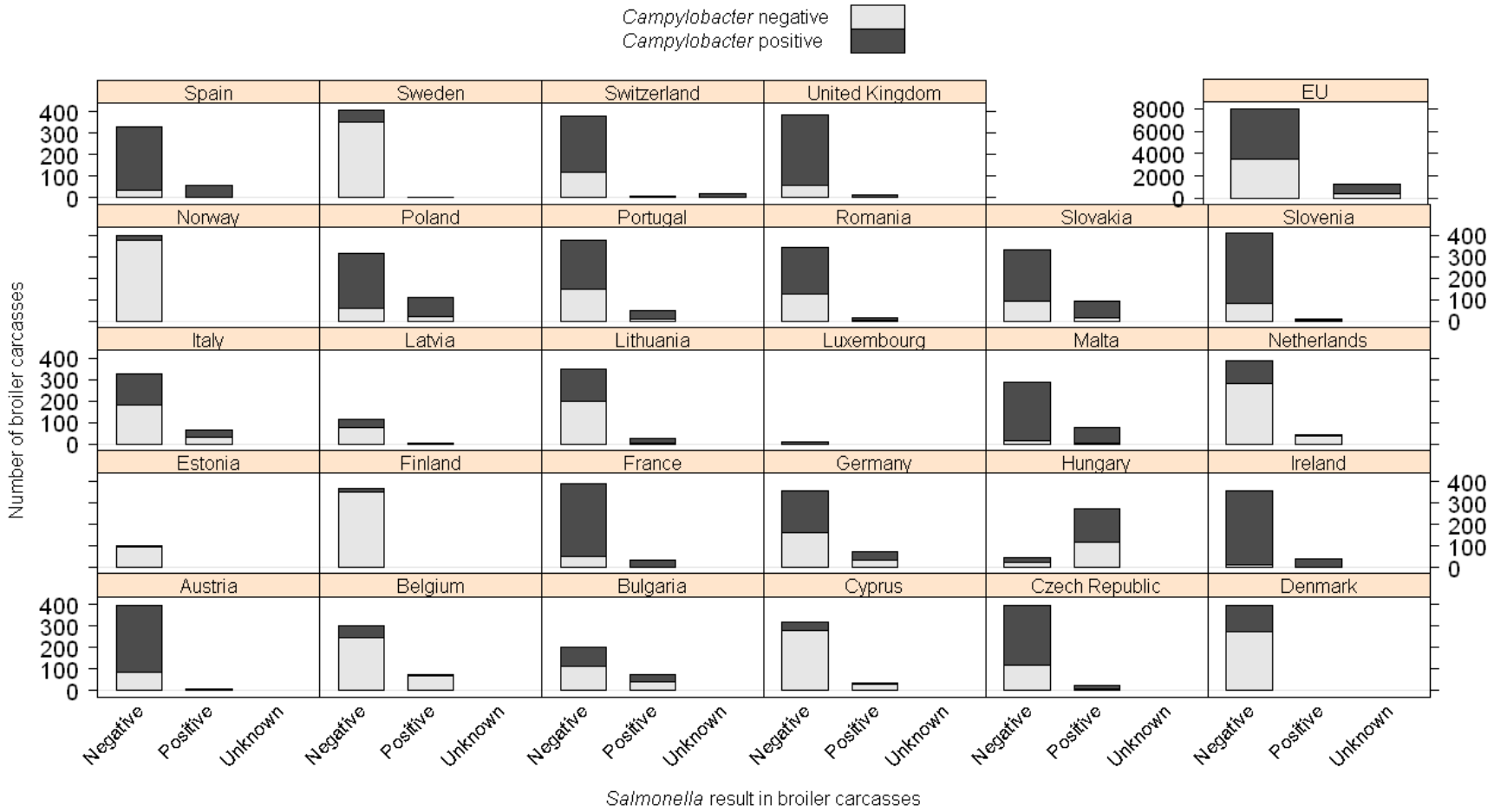

Figure 35. **Multiple bar graphs of estimated (weighted) frequency counts of *Campylobacter*-positive and -negative broiler carcasses by the *Salmonella*-contamination result on the broiler carcass, by country and in the EU*, 2008**

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

G. FINAL MODEL FOR *CAMPYLOBACTER*-CONTAMINATION RESULT ON BROILER CARCASSES: VARIANCE INFLATION FACTOR VALUES AND VARIANCE OF RANDOM EFFECTS

Table 51. **Variance Inflation Factor values for factors potentially related to *Campylobacter*-contaminated broiler carcasses**

Risk Factor	VIF
Flock production type	1.25
Previous thinning of the flock	1.22
Age of broilers	1.48
Quarter of sampling	1.04
Time (hour) of sampling	1.21
Hours between sampling and testing	1.31
Capacity of slaughterhouse	1.28
Type of chilling	1.34
Campylobacter-colonisation result in the broiler batch	1.11
Salmonella-contamination result on the broiler carcass	1.04

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

The variance of the random effects (effect of slaughterhouses) and of the random slopes in the final regression model were both significantly different from zero. The Wald test statistics were 17.2 (*P*-value (<0.001) and 33.3 (*P*-value (<0.001), respectively, and were calculated using a 50:50 mixture of Chi-square distributions with 0 and 1 degrees of freedom, respectively, given that the value under the null hypothesis lies on the border of the parameter space (Molenberghs and Verbeke, 2005). The *P*-value for the covariance was calculated using a Chi-square distribution with 1 degree of freedom (because the values of a covariance are not bounded).

Table 52. **Final logistic mixed-effects model for factors associated with *Campylobacter*-contaminated broiler carcasses, variance of slaughterhouse-specific random intercepts, variance of the random slopes for *Campylobacter*-colonisation result in the broiler batch among slaughterhouses, and the covariance between the random intercepts and the random slopes, in the EU, 2008**

	Estimate	Standard Error	*P*-value
Variance of the random intercepts	2.24	0.54	<0.001
Covariance between the random intercepts and the random slopes	2.12	0.51	<0.001
Variance of the random slopes	4.04	0.70	<0.001

H. FULLS MODEL FOR *CAMPYLOBACTER*-CONTAMINATED BROILER CARCASSES IN COUNTRIES WITH PREVALENCE ABOVE THE EU MEDIAN PREVALENCE AND COUNTRIES WITH PREVALENCE BELOW THE EU MEDIAN PREVALENCE[19]

Table 53. **Comparison of the full models for *Campylobacter* result on broiler carcasses between countries with prevalence below the EU median prevalence and above the EU median prevalence, odds ratio and 95% confidence intervals for the risk factors and *P*-value of the type III test for fixed effects**

Country group	Variable	Level	OR	Lower	Upper	*P*-value
Countries with prevalence below the EU* median prevalence	Flock production type	*Unknown*	1.01	0.43	2.33	0.28
	Reference category: *Conventional*	*Organic*	0.48	0.23	1.01	
		Standard	0.91	0.52	1.59	
	Previous thinning in the flock	*Unknown*	1.14	0.77	1.70	0.451
	Reference category: *No*	*Yes*	1.28	0.87	1.88	
	Age of broilers (scale 10 days)	-	1.15	0.92	1.44	0.205
	Quarter of sampling	*IV*	1.31	0.87	1.99	0.315
	Reference category: *I*	*III*	1.35	0.85	2.13	
		II	1.37	0.97	1.94	
	Time (hour) of sampling during the day	-	1.02	0.98	1.06	0.419
	Capacity of slaughterhouse	-	1.23	0.85	1.80	0.276
	Type of chilling of carcasses	*Mixed*	0.28	0.07	1.08	0.226
	Reference category: *Air*	*Spray*	1.15	0.74	1.78	
		Immersion	1.21	0.73	1.98	
	Campylobacter-colonisation result in the broiler batch Ref: *Negative*	*Positive*	15.15	10.20	22.22	<0.0001
	Salmonella-contamination result on the broiler carcass Ref: *Negative*	*Positive*	1.04	0.72	1.52	0.826
Country group	**Variable**	**Level**	**OR**	**Lower**	**Upper**	***P*-value**
Countries with prevalence above the EU* median prevalence	Flock production type	*Unknown*	2.17	0.67	7.01	<0.0001
	Reference category: *Conventional*	*Organic*	>999.99	210.24	>999.99	
		Standard	0.98	0.36	2.66	
	Previous thinning in the flock	*Unknown*	0.93	0.43	2.01	0.917
	Reference category: *No*	*Yes*	1.08	0.68	1.71	
	Age of broilers (scale 10 days)	-	1.32	1.00	1.75	0.047
	Quarter of sampling	*IV*	1.98	1.21	3.21	0.011
	Reference category: *I*	*III*	1.96	1.24	3.11	
		II	1.32	0.81	2.16	
	Time (hour) of sampling during the day	-	1.05	0.98	1.13	0.159
	Capacity of slaughterhouse	-	0.94	0.52	1.69	0.826
	Type of chilling of carcasses	*Mixed*	3.83	1.38	10.69	0.042
	Reference category: *Air*	*Spray*	1.46	0.54	3.98	
		Immersion	0.89	0.33	2.41	
	Campylobacter-colonisation result in the broiler batch Ref: *Negative*	*Positive*	50	26.32	90.91	<0.0001
	Salmonella-contamination result on the broiler carcass Ref: *Negative*	*Positive*	1.23	0.74	2.05	0.428

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

[19] Two non-MSs, Norway and Switzerland, were included to calculate the EU median prevalence.

I. DESCRIPTIVE ANALYSIS OF POTENTIAL FACTORS ASSOCIATED WITH COUNTS OF *CAMPYLOBACTER* ON CONTAMINATED BROILER CARCASSES

In this appendix a descriptive analysis of the potential factors associated with the counts of *Campylobacter* on contaminated broiler carcasses is provided.

Flock production type

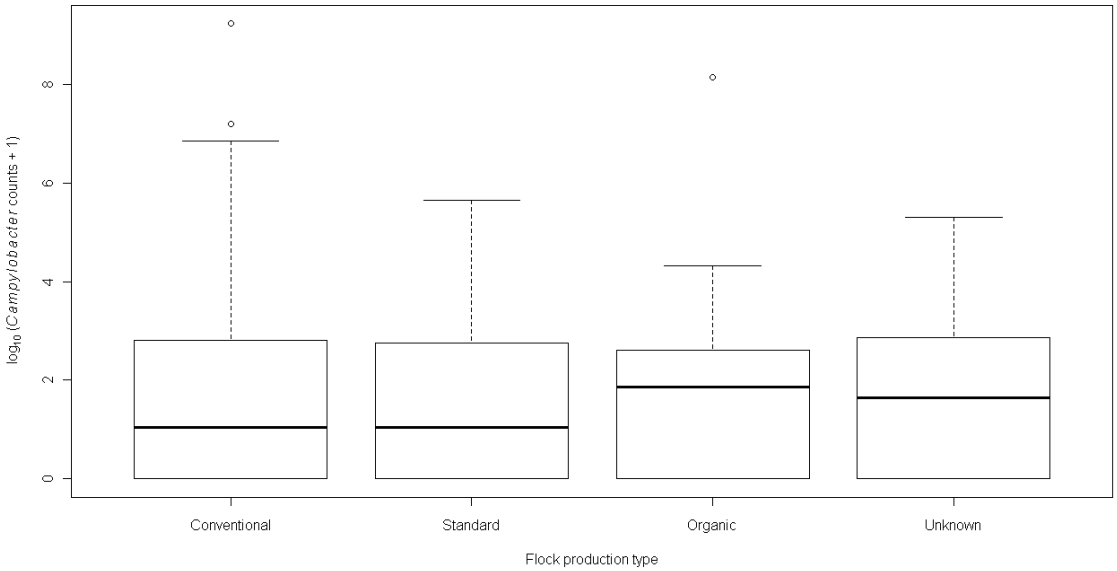

Figure 36. **Boxplot of the $\log_{10}$ (*Campylobacter* counts on broiler carcasses + 1), by flock production type, in the EU*, 2008**

* Exceptionally in Luxembourg no *Campylobacter* enumeration was executed in broiler carcass samples, and Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis. Estonia reported all flocks as free-range standard, but broilers were kept inside with no access to outside and these data are included in the analysis.

In the boxplots, the bottom section of the box represents the first quartile of the distribution and the top section of the box the third quartile, 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). *Campylobacter* counts were added to one previous to $\log_{10}$ transformation in order to allow for the inclusion of negative counts (<10 cfu/g) in these representations.

From Figure 36, it can be observed that the median logarithm of *Campylobacter* counts of conventional and free-range standard flocks are exactly the same (1.04); in fact the main difference between the two groups is a larger variation in the counts for the conventional group. Flock production type is associated with *Campylobacter* counts (considering the categorisation) (Table 54).

Table 54. **Pearson Chi-square test for independence between the flock production type and *Campylobacter* count on the broiler carcass**

	Chi-square statistic (*P*-value)
Campylobacter spp.	78.7 (<0.0001)

Previous thinning in the flock

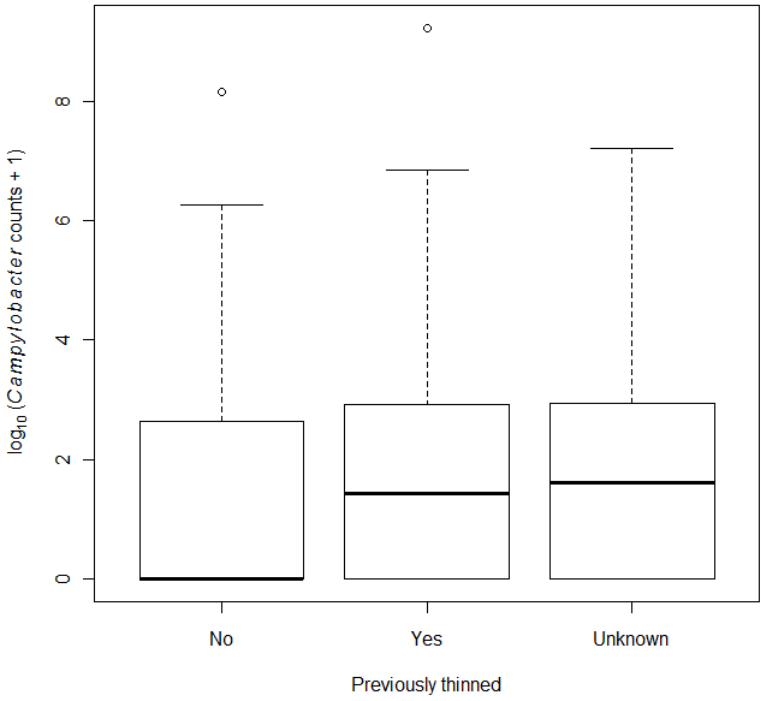

Figure 37. **Boxplot of the $\log_{10}$ (*Campylobacter* counts on broiler carcasses + 1), by previous thinning in the flock, in the EU*, 2008**

* Exceptionally in Luxembourg no *Campylobacter* enumeration was executed in broiler carcass samples, Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

In the boxplots, the bottom section of the box represents the first quartile of the distribution and the top section of the box the third quartile, 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). *Campylobacter* counts were added to one previous to $\log_{10}$ transformation in order to allow for the inclusion of negative counts (<10 cfu/g) in these representations.

The median of the logarithm of *Campylobacter* counts on broiler carcasses is zero in the flocks which were not previously thinned. The medians in the other two categories are very close to each other (Figure 37).

Based on the Pearson Chi-square test, Table 55 there is an association between the counts (considering the categorization) and previous thinning in the flock.

Table 55. **Pearson Chi-square test for independence between previous thinning in the flock and the *Campylobacter* count on the broiler carcass**

	Chi-square statistic (*P*-value)
Campylobacter spp.	178.1 (<0.0001)

Age of broilers

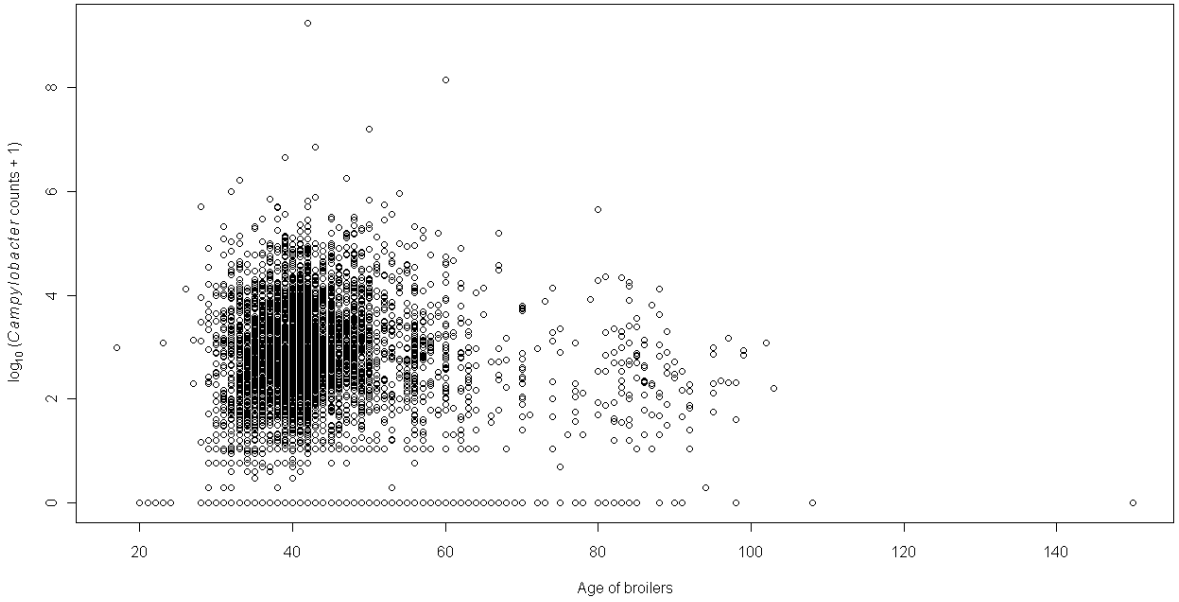

Figure 38. **Scatterplot of the $\log_{10}$ (*Campylobacter* counts on broiler carcasses + 1) and age of broilers, in the EU*, 2008**

* Exceptionally in Luxembourg no *Campylobacter* enumeration was executed in broiler carcass samples, and Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis. *Campylobacter* counts were added to one previous to $\log_{10}$ transformation in order to allow for the inclusion of negative counts (<10 cfu/g) in these representations.

From Table 56, there is small positive linear relationship between the results of *Campylobacter* based on the enumeration test and the age of broilers. In fact, Figure 38 does not reveal any relational pattern.

Table 56. **Spearman correlation coefficient to test linear association between age of broilers and *Campylobacter* count on the broiler carcass**

	Spearman correlation (*P*-value)
Campylobacter spp.	0.19 (<0.0001)

Quarter of sampling

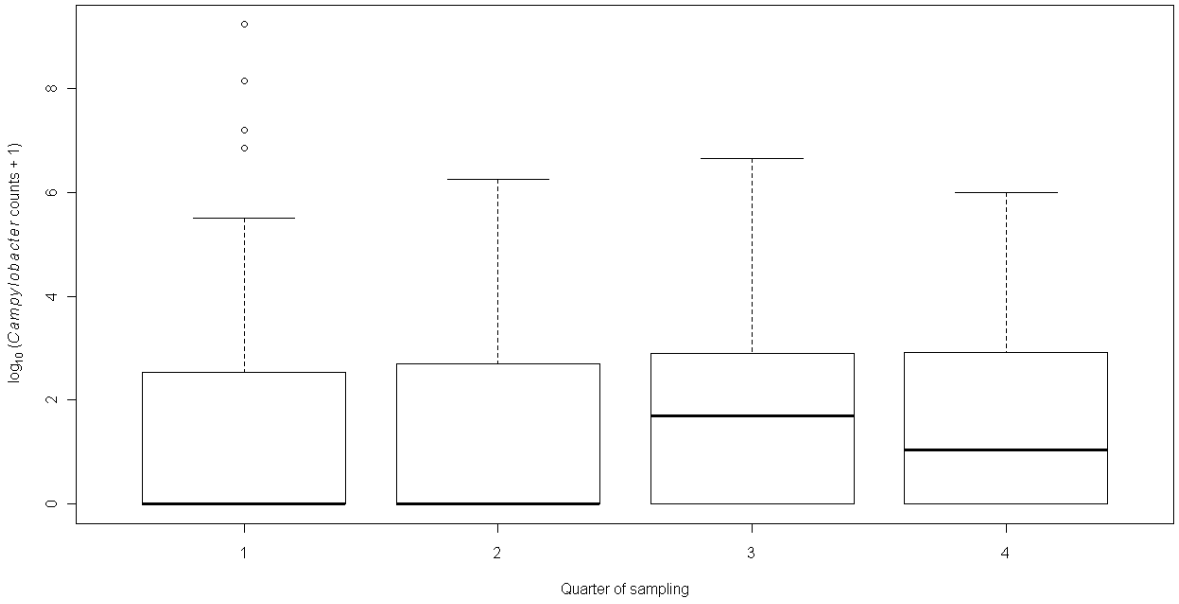

Figure 39. **Boxplot of the $\log_{10}$ (*Campylobacter* counts on broiler carcasses + 1), by quarter of sampling, in the EU*, 2008**

* Exceptionally in Luxembourg no *Campylobacter* enumeration was executed in broiler carcass samples, and Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

In the boxplots, the bottom section of the box represents the first quartile of the distribution and the top section of the box the third quartile, 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). *Campylobacter* counts were added to one previous to $\log_{10}$ transformation in order to allow for the inclusion of negative counts (<10 cfu/g) in these representations.

The median logarithm of the counts is much bigger in the third quarter, which agrees with previous results about the highest prevalence of *Campylobacter* in this quarter of sampling (Figure 39).

In order to assess the association between the categorical outcomes and the counts, the counts were categorised as follows: 0-9 cfu/g, 10-39 cfu/g, 40-99 cfu/g, 100-999 cfu/g, 1000-10000 cfu/g, and >10000 cfu/g. Then a Pearson Chi-square test was used (Table 57) to test for independence between quarter of sampling and *Campylobacter* count on the broiler carcass.

Table 57. **Pearson Chi-square test for independence between quarter of sampling and *Campylobacter* count on the broiler carcass**

	Chi-square correlation (*P*-value)
Campylobacter spp.	201.2 (<0.0001)

Time (hour) of sampling

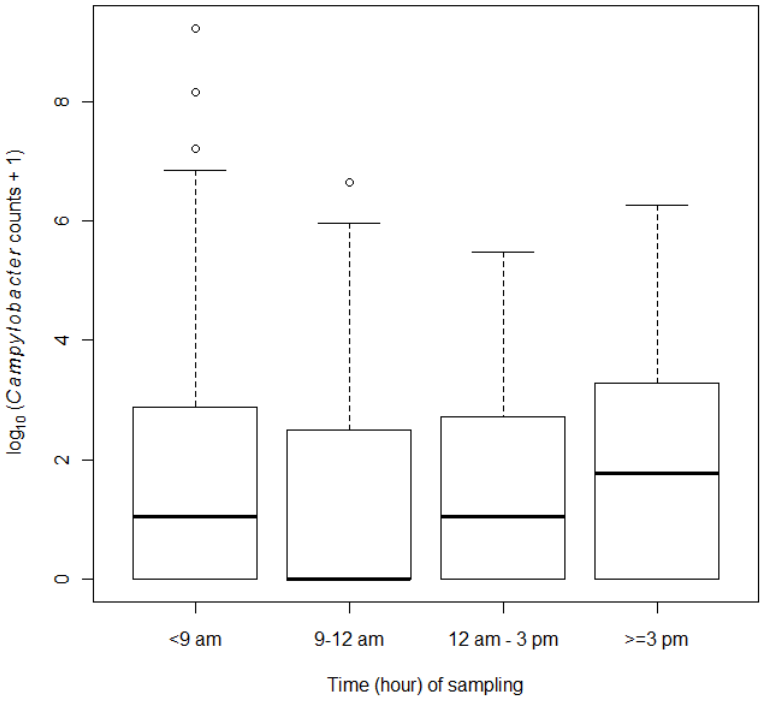

Figure 40. **Boxplot of the $\log_{10}$ (*Campylobacter* counts on broiler carcasses + 1), by time (hour) of sampling, in the EU*, 2008**

* Exceptionally in Luxembourg no *Campylobacter* enumeration was executed in broiler carcass samples, and Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

In the boxplots, the bottom section of the box represents the first quartile of the distribution and the top section of the box the third quartile, 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). *Campylobacter* counts were added to one previous to $\log_{10}$ transformation in order to allow for the inclusion of negative counts (<10 cfu/g) in these representations.

In this case, the association between the time (hour) of sampling during the day and the result of the *Campylobacter* test based on the enumeration method is not significant (Table 58).

Table 58. **Spearman correlation coefficient to test association between time (hour) of sampling during the day and *Campylobacter* count on the broiler carcass**

	Spearman correlation (*P*-value)
Campylobacter spp.	-0.008 (0.40)

Hours between sampling and testing

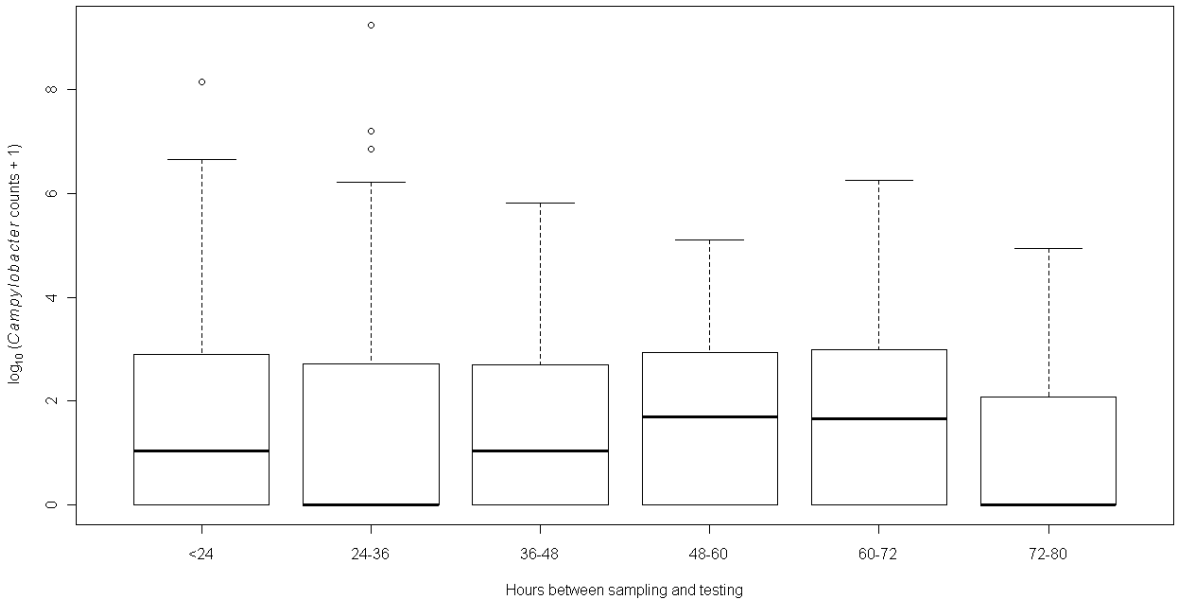

Figure 41. **Boxplot of the $\log_{10}$ (*Campylobacter* counts on broiler carcasses + 1), by hours between sampling and testing, in the EU*, 2008**

* Exceptionally in Luxembourg no *Campylobacter* enumeration was executed in broiler carcass samples, and Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

In the boxplots, the bottom section of the box represents the first quartile of the distribution and the top section of the box the third quartile, 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). *Campylobacter* counts were added to one previous to $\log_{10}$ transformation in order to allow for the inclusion of negative counts (<10 cfu/g) in these representations.

Figure 41 shows very similar distribution of the *Campylobacter* counts within the categories of hours between sampling and testing. This observation is confirmed by the Spearman correlation coefficient (Table 59).

Table 59. **Spearman correlation coefficient to test association between hours between sampling and testing and *Campylobacter* counts on the broiler carcass**

	Spearman correlation (*P*-value)
Campylobacter spp.	0.00015 (0.99)

Capacity of slaughterhouse

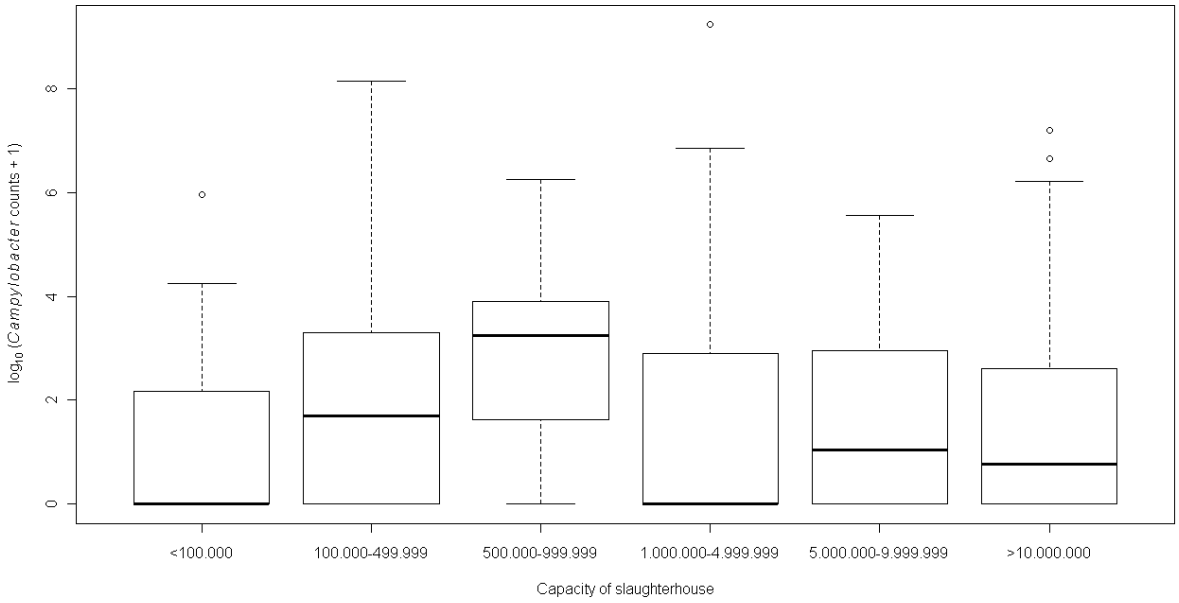

Figure 42. **Boxplot of the $\log_{10}$ (*Campylobacter* counts on broiler carcasses + 1), by slaughterhouse capacity, in the EU*, 2008**

* Exceptionally in Luxembourg no *Campylobacter* enumeration was executed in broiler carcass samples, and Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.)

In the boxplots, the bottom section of the box represents the first quartile of the distribution and the top section of the box the third quartile, 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). *Campylobacter* counts were added to one previous to $\log_{10}$ transformation in order to allow for the inclusion of negative counts (<10 cfu/g) in these representations.

The Spearman correlation coefficient indicates a very small, but significant, association between the capacity of the slaughterhouse and the result of *Campylobacter* enumeration method (Table 60).

Table 60. **Spearman correlation coefficient to test association between capacity of the slaughterhouse and *Campylobacter* count on the broiler carcass**

	Spearman correlation (*P*-value)
Campylobacter spp.	-0.087 (<0.0001)

Type of chilling

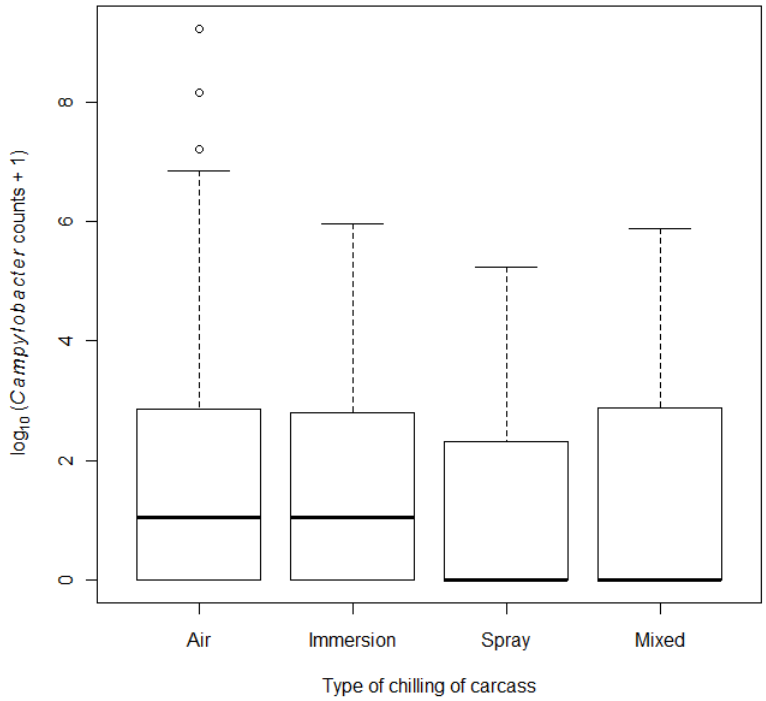

Figure 43. **Boxplot of the $\log_{10}$ (*Campylobacter* counts on broiler carcasses + 1), by type of carcass chilling, in the EU*, 2008**

* Exceptionally in Luxembourg no *Campylobacter* enumeration was executed in broiler carcass samples, and Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

In the boxplots, the bottom section of the box represents the first quartile of the distribution and the top section of the box the third quartile, 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). *Campylobacter* counts were added to one previous to $\log_{10}$ transformation in order to allow for the inclusion of negative counts (<10 cfu/g) in these representations.

Figure 43, displays the boxplot of the logarithm of *Campylobacter* counts (considering the categorisation) within the categories of the type of carcass chilling. The medians in the first two groups, chilling of the flock by air or by immersion are equal to 1.04 and in the other two categories, spray and mixed are equal to zero.

Table 61. **Pearson Chi-square test for independence between the type of chilling and *Campylobacter* count on the broiler carcass**

	Chi-square statistic (*P*-value)
Campylobacter spp.	190.4 (<0.0001)

***Campylobacter*-colonisation result in the broiler batch**

Table 62. **Pearson Chi-square test for independence between the *Campylobacter*-colonisation result in the broiler batch and the *Campylobacter* count on the broiler carcass**

	Chi-square statistic (*P*-value)
Campylobacter spp.	3607.2 (<0.0001)

***Salmonella*-contamination result on the broiler carcass**

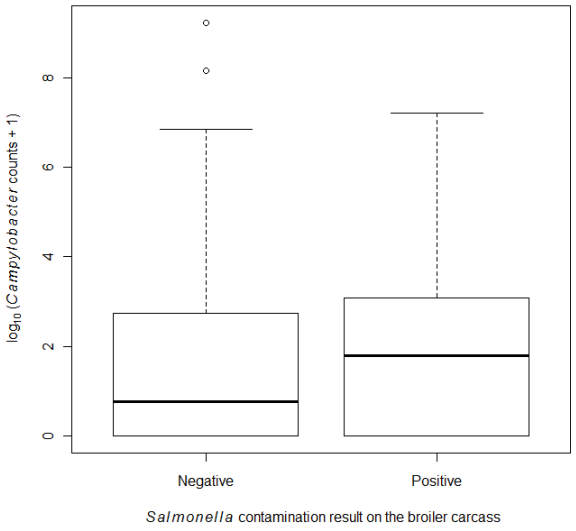

Figure 44. **Boxplot of the $\log_{10}$(*Campylobacter* counts on broiler carcasses + 1), by *Salmonella*-contamination result on the broiler carcass**

* Exceptionally in Luxembourg no *Campylobacter* enumeration was executed in broiler carcass samples, Greece did not participate in the baseline survey and two non-MSs, Norway and Switzerland, participated and are included in this analysis.

In the boxplots, the bottom section of the box represents the first quartile of the distribution and the top section of the box the third quartile, 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). *Campylobacter* counts were added to one previous to $\log_{10}$ transformation in order to allow for the inclusion of negative counts (<10 cfu/g) in these representations.

Table 63. **Pearson Chi-square test for independence between the *Salmonella*-contamination result on the broiler carcass and the *Campylobacter* count on the broiler carcass**

	Chi-square statistic (*P*-value)
Campylobacter spp.	31.0 (<0.0001)

J. ANALYSIS OF THE VARIANCE COMPONENTS

In order to investigate the importance of the slaughterhouse-specific effects (random variable effect) the variance components of the multivariable models, i.e. the between-slaughterhouse variability and the within-slaughterhouse variability, were quantified. Calculation of the between-slaughterhouse variability (due to slaughterhouse-specific effects) was calculated based on the ICC, which describes the similarity of the responses on the outcome within a slaughterhouse (cluster). The ICC ranges between 0 and 1. A high ICC, close to one, means that the probability of carcass contamination is more influenced by factors operating at slaughterhouse-level. Lower ICC values, close to zero, correspond to the reverse situation: the outcomes are more influenced by factors operating at broiler's batch-level.

For a random-effects model, the ICC considers the variance of the random effects and the variance of the standard logistic density (Molenberghs and Verbeke, 2005) and is calculated as follows:

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

where:

- z is a matrix of estimable functions, and
- D is the unstructured variance-covariance matrix of the random effects b_i.

Moreover, the ICC effect was also investigated based on the regression outcome regarding the prevalence of *Campylobacter*-contaminated broiler carcasses. The ICCs in the two groups, namely, the group with positive (ICC_1) and the group with negative (ICC_0) *Campylobacter*-colonisation results in the broiler batch, were calculated as follows:

$$ICC_0 = \frac{\sigma_0^2}{\sigma_0^2 + \pi^2/3} \qquad ICC_1 = \frac{\sigma_0^2 + 2\sigma_{01} + \sigma_1^2}{\sigma_0^2 + 2\sigma_{01} + \sigma_1^2 + \pi^2/3}.$$

K. FINAL MODEL FOR *CAMPYLOBACTER* COUNTS ON CONTAMINATED BROILER CARCASSES, AND STANDARD ERRORS FOR THE INTERCEPTS FOR EACH COUNTRY

Table 64. **Negative binomial model for *Campylobacter* counts greater than 10 cfu/g on broiler carcasses: estimates of the fixed effects per country with *P*-value of the Wald's test**

Country	Estimate	Standard Error	*P*-value
Austria	8.06	0.41	<.0001
Belgium	7.75	0.35	<.0001
Bulgaria	8.93	0.34	<.0001
Cyprus	6.44	0.52	<.0001
Czech Republic	7.91	0.34	<.0001
Denmark	7.04	0.39	<.0001
Estonia	8.86	0.06	<.0001
Finland	5.25	0.80	<.0001
France	5.49	0.27	<.0001
Germany	7.03	0.47	<.0001
Hungary	7.17	0.39	<.0001
Ireland	7.28	0.31	<.0001
Italy	6.28	0.42	<.0001
Latvia	5.28	0.28	<.0001
Lithuania	6.75	0.60	<.0001
Malta	9.18	0.23	<.0001
Netherlands	7.03	0.31	<.0001
Norway	2.42	0.38	<.0001
Poland	6.70	0.26	<.0001
Portugal	5.92	0.52	<.0001
Romania	8.09	0.25	<.0001
Slovakia	7.78	0.41	<.0001
Slovenia	5.32	0.34	<.0001
Spain	7.70	0.31	<.0001
Sweden	4.91	0.32	<.0001
Switzerland	7.66	0.30	<.0001
United Kingdom	6.81	0.36	<.0001

Table 64 shows the estimates of the fixed effects for each country. These parameter estimates are proportional to the counts of *Campylobacter* in the country. Indeed, keeping fixed the other covariates in the model, the higher the parameter estimate, the higher the counts in that country.

L. ANALYSIS OF *CAMPYLOBACTER* SPECIES FREQUENCY DISTRIBUTION

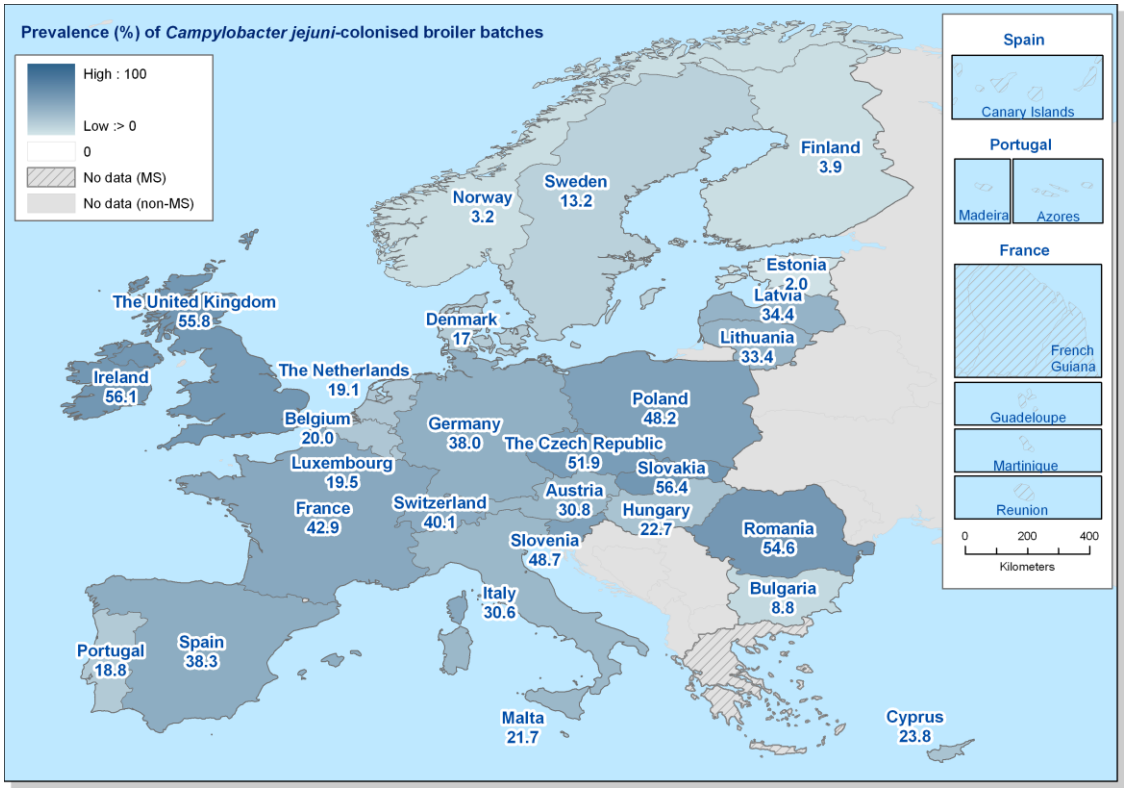

Figure 45. **Prevalence of *Campylobacter jejuni*-colonised broiler batches**

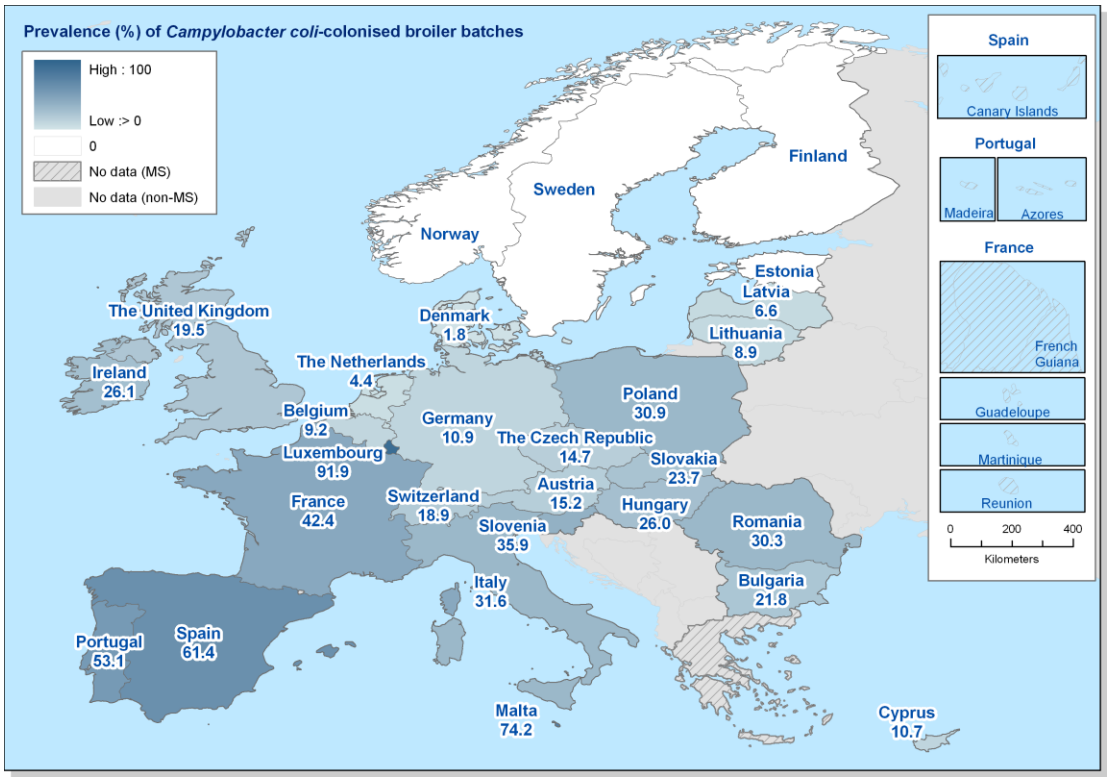

Figure 46. **Prevalence of *Campylobacter coli*-colonised broiler batches**

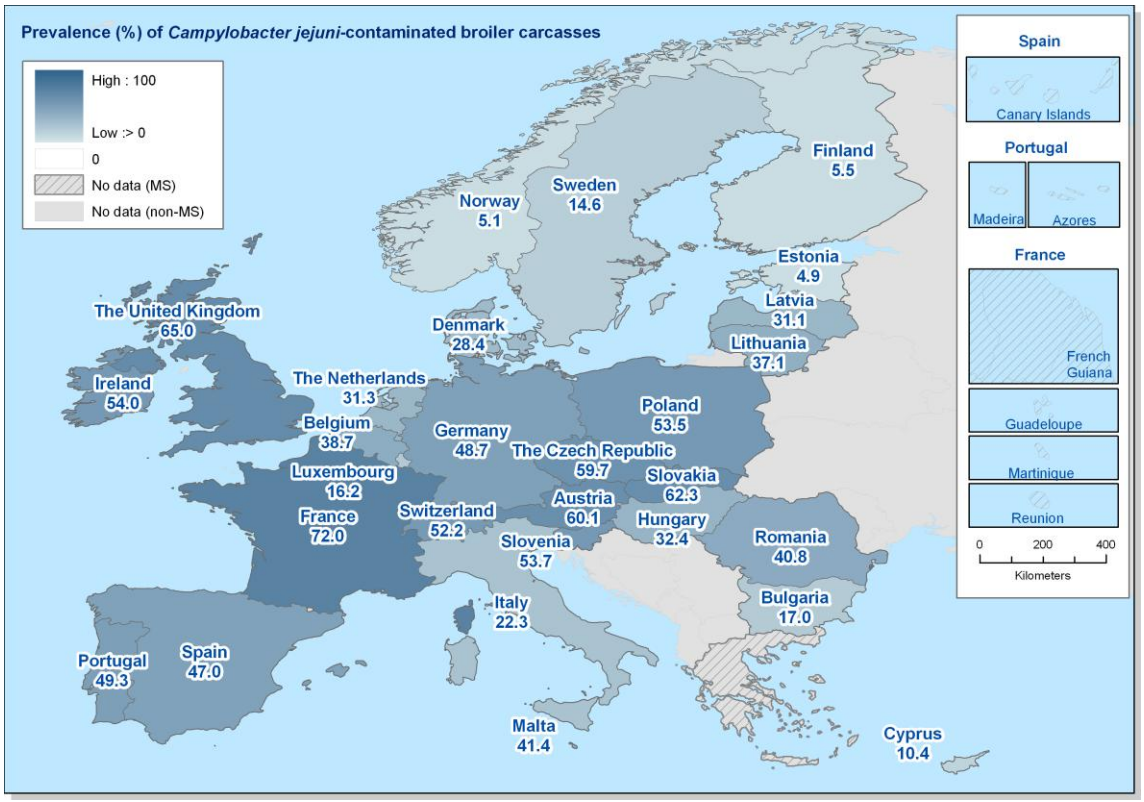

Figure 47. **Prevalence of *Campylobacter jejuni*-contaminated broiler carcasses**

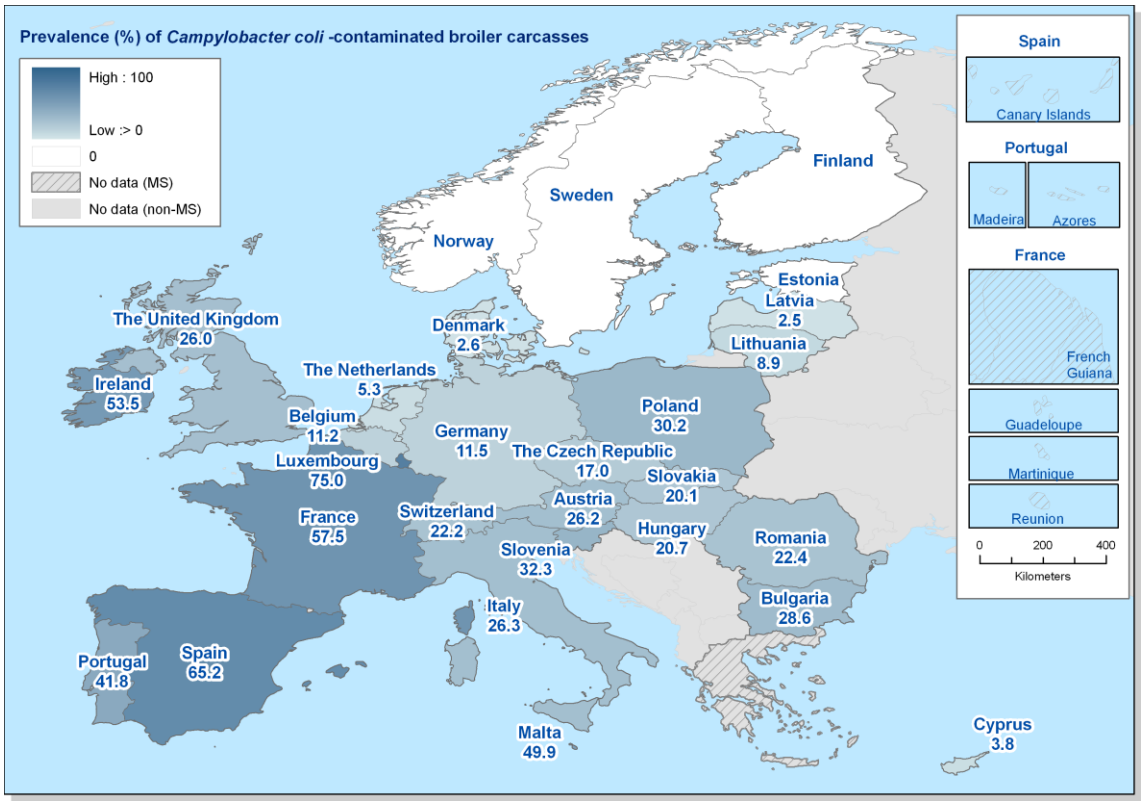

Figure 48. **Prevalence of *Campylobacter coli*-contaminated broiler carcasses**

ABBREVIATIONS

cfu	colony forming units
CI(s)	Confidence Interval(s)
DNA	Deoxyribonucleic acid
EC	European Commission
EFSA	European Food Safety Authority
ESBL	Extended-Spectrum-Beta-Lactamase
EU	European Union
ICC	Intra-cluster Correlation Coefficient
ISO	International Organization for Standardization
mCCDA	modified charcoal cefoperazone deoxycholate agar
MS(s)	Member State(s)
OR(s)	Odds Ratio(s)
PCR	Polymerase Chain Reaction
VIF	Variance Inflation Factor