# Report of the Task Force on Zoonoses Data Collection on the Analysis of the baseline study on the prevalence of *Salmonella* in holdings of laying hen flocks of *Gallus gallus*[1]

**(Question N° EFSA-Q-2006-039)**

**Adopted by**
**The Task Force on 20 February 2007**

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# Summary

*Salmonella* is an important cause of foodborne disease in humans throughout the world and is a significant cause of morbidity, mortality and economic loss. Hens' eggs which are derived from flocks infected with *Salmonella* Enteritidis are an important source of this serovar. *S.* Enteritidis is the serovar which causes more than 50% of human infections with *Salmonella* in the European Union[1]. The second most reported serovar in humans is *S.* Typhimurium, which is less often associated with the consumption of hens' eggs.

The European Union has agreed a programme for the reduction of *Salmonella* of public health significance in farm animals under Regulation EC No 2160/2003. In order to provide the scientific basis for setting targets for *Salmonella* in laying flocks of *Gallus gallus*, a European Union-wide baseline study to determine the prevalence of *Salmonella* was conducted on commercial large-scale laying hen holdings with at least 1,000 laying hens on the holding. This study was the first of several baseline studies organised at the European Community level.

The sampling of the holdings took place between October 2004 and September 2005. Five faeces and two dust samples were taken from flocks of laying hens during the last nine weeks of their production. A total of 5,310 holdings with validated results were included in the study analyses.

*Salmonella* was detected in 30.8% of the laying hen holdings in the European Union. In the specific Member States, the observed holding prevalence of *Salmonella* ranged from 0% to 79.5%. A total of 20.4% of the laying hen holdings was positive for *S.* Enteritidis / *S.* Typhimurium. The Member State-specific observed holding prevalence of *S.* Enteritidis / *S.* Typhimurium varied greatly, from 0% to 62.5%. The prevalence of *Salmonella,* especially *S.* Enteritidis, was greater than that predicted by existing routine surveillance in most Member States.

Due to the design of the study, which resulted from the pragmatic decision to sample only one flock per holding, the true holding prevalence is likely to be higher than the observed, as some of the holdings detected negative may house one or more positive flocks that were not sampled and hence not detected. Moreover, the design of the study did not allow the flock prevalence to be estimated without additional information.

The number of positive samples in a *Salmonella* positive holding varied between one and seven but 38% of those positive holdings was found positive on the basis of only one or two *Salmonella* positive samples.

The three most frequently isolated *Salmonella* serovars in the European Union were *S.* Enteritidis, *S.* Infantis and *S.* Typhimurium. *S.* Enteritidis was by far the most common serovar and it was detected in 60% of the *Salmonella* positive holdings.

Vaccination of the hens in the flock against *Salmonella* was associated with a lower risk of being *Salmonella* positive, except for holdings infected with *S.* Typhimurium. In Member States with an intermediate *S.* Enteritidis holding prevalence (2.5%-15%), vaccination also seemed less important for the *S.* Enteritidis status of the holding.

Cage production was found to be associated with a higher risk of positivity than for the other investigated laying hens production types. However, compared to the other production types, cage production was characterised by larger flock sizes. Organic flocks were on average of the smallest size, whereas the barn and the free-range standard flocks were of low to medium size. Consequently cage production as well as a larger flock size were associated with a higher risk of positivity. But it was not possible to determine which of these two factors was a true risk factor for positivity.

[1]The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2005, *The EFSA Journal* (2006) 94.

Potential factors associated with prevalence of serovars other than *S.* Enteritidis and *S.* Typhimurium were numerous, including the seasonality.

There were indications that factors associated with *Salmonella* prevalence may depend on the specific *Salmonella* serovar epidemiology. *S.* Enteritidis and *S.* Typhimurium showed no evidence of seasonal variation, whereas serovars other than *S.* Enteritidis and *S.* Typhimurium peaked in autumn months. There were also differences between the factors associated with *S.* Enteritidis positivity and those associated with *S.* Typhimurium positivity.

Overall, dust samples were twice more likely to be positive than faeces samples, indicating that sampling of dust is a more sensitive method for detecting *Salmonella* in a laying flock environment.

The phage typing and antimicrobial susceptibility testing information reported was not representative of the whole of the European Union. The distribution of reported *S.* Enteritidis phage types resembled that of human *S.* Enteritidis infections. The observed proportions of *Salmonella*-positive laying hen holdings with resistant isolates were in general low.

In the future baseline studies, improved validation during the data submission period would streamline the reporting and analyses. Also making the reporting on antimicrobial resistance and phage typing obligatory would provide for more representative information. Further studies on risk factors are needed to confirm the results regarding factors related to *Salmonella* positivity.

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# 1. Introduction

In order to provide the scientific basis for setting targets for the prevalence of *Salmonella* in laying flocks of *Gallus gallus*, a European Union-wide *Salmonella* baseline study to determine the prevalence of *Salmonella* was conducted on a randomised selection of commercial large-scale laying hen holdings with at least 1,000 laying hens on the holding [1]. This study was the first of several baseline studies organised at the European Community level.

The study was carried out between 1 October 2004 and 30 September 2005, and in accordance with the Community legislation on zoonoses aiming at reducing the incidence of foodborne diseases in the European Union (EU). Regulation EC No 2160/2003 on the control of *Salmonella* and other specified zoonotic agents[2] foresees the setting of a Community target for reducing *Salmonella* prevalence in laying hens. Therefore, a baseline study was carried out to support the setting of such a target by obtaining comparable information on the prevalence of *Salmonella* in laying hen flocks in the EU Member States (MSs). Norway also participated in the study on a voluntary basis.

The objectives, the sampling frame and the diagnostic testing methods, as well as the collection of data, evaluation, reporting and timelines of this baseline study are specified in Commission Decision 2004/665/EC concerning a baseline study on the prevalence of *Salmonella* in laying flocks of *Gallus gallus*[3].

A Preliminary Report on the Analysis of the baseline study on the prevalence of *Salmonella* in laying hen flocks of *Gallus gallus*[4] was published by The European Food Safety Authority (EFSA) on 14 June 2006. This Preliminary Report describes some of the results of the baseline study and reports the observed prevalence of *Salmonella*-positive holdings in the EU and in the MSs based on the dataset as it was on 24 February 2006. After the publication of this Preliminary Report, MSs were given the opportunity to correct their data and a new revised dataset was submitted to the European Commission.

[1] Commission Decision of 22 September 2004 concerning a baseline study on the prevalence of *Salmonella* in laying flocks of *Gallus gallus*. 2004/665/EC. *Official Journal of the European Union* 2004; L303/30: 30.9.2004.
[2] 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. *Official Journal of the European Union* 2003; L 325/1: 12.12.2003.
[3] Commission Decision of 22 September 2004 concerning a baseline study on the prevalence of *salmonella* in laying flocks of *Gallus gallus*. 2004/665/EC. *Official Journal of the European Union* 2004; L303/30: 30.9.2004.
[4] *The EFSA Journal* (2006) 81, 1-71 (http://www.efsa.europa.eu/en/science/monitoring_zoonoses/reports/1541.html)

# 2. Objectives

The aim of the study was to estimate the observed prevalence of *Salmonella*-positive holdings amongst commercial large-scale holdings (i.e. holdings containing at least 1,000 laying birds) of laying hens across the EU, at the whole EU level as well as for each MS.

The specific objectives were:

- to estimate the holding prevalence of *Salmonella* in commercial large-scale holdings of laying hens at the EU level and each MS specifically,
- to estimate the holding prevalence of the two serovars, *Salmonella* Enteritidis and *Salmonella* Typhimurium for, pursuant to article 4 of the Regulation EC No 2160/2003, the *Salmonella* reduction target should cover for a provisional period at least these two serovars,
- to investigate the serovar distribution and determine the most frequently occurring serovars in laying hen holdings across the EU,
- to investigate the effect of potential risk factors, such as *Salmonella*-vaccination status, number of birds per holding, and time of sampling, which may be associated with the occurrence of *Salmonella*,
- to evaluate the sampling design especially with regard to the precision and accuracy of the prevalence estimates.

Member States were also invited to submit additional information on *S.* Enteritidis and *S.* Typhimurium phage types and antimicrobial susceptibility of *Salmonella* isolates, but this testing was not a compulsory requirement of the survey.

# 3. Materials and methods

The sampling scheme of the baseline study is prescribed in the document European Commission DG SANCO: Baseline study on the prevalence of *Salmonella* in laying flocks of *Gallus gallus* in the EU: Technical specifications. SANCO/34/2004 Rev3[1].

A detailed description of the design of the baseline study, the sample size and the bacteriological testing is found in the Preliminary Report. Samples were taken from flocks of laying hens during the last nine weeks of the laying period. In each randomly selected holding, one flock was sampled by taking five large naturally pooled faeces samples (cage flocks[2]), five pairs of 'bootsock' swabs (barn[3] or free-range[4] flocks) and two large dust samples. The number of holdings to be sampled was stratified according to the holding size, meaning that a certain number of holdings in different size categories of holdings had to be sampled. Samples were taken by agents of the Competent Authority in each MS and were tested by the National Reference Laboratory (or a laboratory authorised by it) using the new ISO 6579 Annex D method. Phage typing was carried out according to Colindale schemes, and antimicrobial susceptibility testing was carried out by the normal method for the MS.

## 3.1. Data description

### 3.1.1. Data validation and cleaning

EFSA received the final dataset from the European Commission on 15 May 2006. This dataset contained data from 5,351 laying-hen holdings in 24 MSs and from 314 holdings in Norway. The dataset did not include data from Malta.

A set of data exclusion criteria (Annex I) was used to identify non-valid and non-plausible information in the dataset. This resulted in a cleaned, validated dataset comprising 5,310 holdings which formed the basis for all subsequent analyses. An overview of the number of holdings per country included in the validated dataset is given in Annex II. This dataset did not include Slovakian data since all records from this MS were excluded. All together, 6% of the holdings (355 out of 5,665) was excluded from the full dataset. Compared to the cleaned dataset presented in the "Preliminary report", this final cleaned dataset contained data from 446 additional holdings from the MSs and 67 additional holdings from Norway.

An overview of the number of excluded holdings per MS is given in Table 1. The reasons for exclusion of samples or holdings in accordance with the exclusion criteria are summarized in Annex III. The criterion that caused the highest number of records to be excluded was a discrepancy greater than 10% between the reported number of hens in the holding and the reported

[1] European Commission DG SANCO. Baseline study on the prevalence of *Salmonella* in laying flocks of Gallus gallus in the European Union: Technical specifications. SANCO/34/2004 Rev3. Working document, 13 July 2004. Presented at the meeting of the Standing Committee on the Food Chain and Animal Health on 15 July 2004. (http://europa.eu.int/comm/food/food/biosafety/salmonella/tech_spec_sanco-34-2004_rev-3_en.pdf)

[2] A cage flock has a production type that consists of tiers of cages in which hens are housed together. The cages have sloping mesh floors so that the eggs roll forward, out of the reach of the birds, to await collection.

[3] In the barn production type the hens are kept in loose flocks confined within a shed. Birds in this system are not caged and can roam throughout their house but are not let outside.

[4] A free-range flock system is a flock production type where the birds are housed as in the barn system. But in addition the birds must have continuous daytime access to open runs, which are mainly covered with vegetation.

number of hens in the flock for holdings with only one reported flock. Some records had more than one non-plausible data characteristic.

**Table 1. Overview of the data validation, *Salmonella* in laying hens holding baseline study in the EU, 2004-2005**

| Member States | Number of holdings structurally validated by COM and sent to EFSA | Number of holdings validated on contents by EFSA | Number of holdings excluded by EFSA |
|---|---|---|---|
| | Full dataset | Clean dataset | |
| Austria | 352 | 337 | 15 |
| Belgium | 149 | 141 | 8 |
| Cyprus | 25 | 25 | 0 |
| Czech Republic | 70 | 64 | 6 |
| Denmark | 187 | 185 | 2 |
| Estonia | 11 | 11 | 0 |
| Finland | 269 | 250 | 19 |
| France | 524 | 511 | 13 |
| Germany | 563 | 553 | 10 |
| Greece | 155 | 140 | 15 |
| Hungary | 277 | 267 | 10 |
| Ireland | 156 | 146 | 10 |
| Italy | 381 | 367 | 14 |
| Latvia | 6 | 6 | 0 |
| Lithuania | 11 | 9 | 2 |
| Luxembourg | 9 | 9 | 0 |
| Poland | 362 | 328 | 34 |
| Portugal | 86 | 44 | 42 |
| Slovakia | 33 | 0 | 33 |
| Slovenia | 108 | 98 | 10 |
| Spain | 507 | 485 | 22 |
| Sweden | 185 | 168 | 17 |
| The Netherlands | 471 | 409 | 62 |
| The United Kingdom | 454 | 454 | 0 |
| EU | 5,351 | 5,007 | 344 |
| Norway | 314 | 303 | 11 |

## 3.2. Analysis of data

### 3.2.1. Estimation of the *Salmonella* prevalence

The estimation of the holding prevalence was done for different groups of *Salmonella* serovars as follows:

- Observed holding prevalence of *Salmonella* spp., i.e. all *Salmonella* serovars,
- Observed holding prevalence of *S.* Enteritidis and/or *S.* Typhimurium ('*S.* Enteritidis and/or *S.* Typhimurium'),
- Observed holding prevalence of *S.* Enteritidis,
- Observed holding prevalence of *S.* Typhimurium, and
- Observed holding prevalence of serovars other than *S.* Enteritidis and *S.* Typhimurium.

A holding was considered positive if the presence of *Salmonella* spp. or the specific serovar was detected in at least one of the seven samples taken. For data analysis, a logistic regression model was implemented with *Salmonella*- positivity collapsed at the holding- level.

The observed holding prevalence at EU and at MS-specific level was estimated by accounting for the characteristics of the study data provided by the study design. At EU level, the study design was interpreted as a stratified sampling design with unequal sampling probabilities in each MS, each representing a stratum. Indeed, the proportion of holdings sampled in each MS differed and therefore appropriate weights needed to be given to the MS-specific figures when estimating the EU prevalence. The population of laying hen holdings was considered finite. When estimating the prevalence of the finite population, a correction factor was applied taking account of the number of laying hen holdings in each MS. The 95% confidence intervals (CIs) for the observed prevalence of *Salmonella*-positive holdings were estimated by linear interpolation on the basis of the normalised cumulative probability of 0.975.

Analyses were performed using the statistical software Stata®/SE 9.1 (StataCorp LP, Texas, USA, 2006).

### 3.2.2. Sensitivity analysis of the study design

#### 3.2.2.1 Design-bias

The study design prescribed that only one flock per holding should be sampled. However, by doing this, the observed holding prevalence estimates are most likely underestimated, since some of the observed negative multi-flock holdings may actually house one or more undetected positive flocks and are consequently misclassified. The degree of misclassification depends on the *Salmonella* intra-holding correlation coefficient which reflects the likelihood of flocks in the same holding being *Salmonella*-positive.

Unfortunately, this factor is unknown, but is likely to vary between holdings, regions and/or countries. So in order to investigate the impact of the design-bias on the *Salmonella* prevalence, a simulation was carried out using a range of values (0.0, 0.05, 0.1, …, 1.0) for the *Salmonella* intra-holding correlation coefficient. The simulation exercise randomly recoded negative multi-flock

holdings as positive, based on a chance of 'one minus the value of the assumed *Salmonella* intra-holding correlation coefficient'.

#### 3.2.2.2 Number and type of samples taken in the flock

In order to assess the robustness of the data analysis results, a second simulation exercise was set up to explore how the estimated EU observed prevalence of *S.* Enteritidis-positive holdings would change if a combination of numbers and types of samples other than 5 faeces samples and 2 dust samples had been used. As *S.* Enteritidis was currently the most prevalent serovar in laying hen holdings it was decided to implement the simulation exercise based on this serovar alone.

On the basis of the clean dataset, the number of positive samples in a hypothetical trial (with varying numbers of samples) was modelled as a beta-binomial process (modelled separately for faeces samples and dust samples). The beta distribution was used to model the uncertainty around the proportion of positive faeces and dust samples, while the binomial distribution modelled the variability in the number of positive samples in a hypothetical baseline study with another number of samples. As in the actual baseline survey, in this simulation a holding was considered positive if at least one of the (simulated) samples from the holding was positive. The simulation also assumed that there was no design bias and that there was no error in considering multi-flock holdings negative when the tested flock was negative.

At every iteration the number of *S.* Enteritidis infected holdings was counted. The number of *S.* Enteritidis infected holdings in the total number of holdings in a country was then estimated by Bayesian inference (hyper-geometric likelihood, uniform prior), and on the basis of these simulated data the EU observed prevalence of *S.* Enteritidis-positive holdings was calculated.

The simulation results for different combinations of numbers and types of sample were summarised as uncertainty distributions of the EU observed prevalence of *S.* Enteritidis-positive holdings.

The simulation was implemented using the software @RISK® 4.5.5 (Palisade Corporation, 2004).

### 3.2.3. Analysis of factors associated with *Salmonella* prevalence

With the aim of analysing factors potentially associated with the *Salmonella* prevalence at the EU level and for the MS-groups to have comparable observed holding prevalence figures, the validated dataset was further divided into three subsets. The subsets were used to analyse the association between potential risk factors and the prevalence of *S.* Enteritidis, *S.* Typhimurium and of other serovars than *S.* Enteritidis and *S.* Typhimurium. To this end, holdings with an unknown vaccination status or flock production type, or holdings with samples not complying with the technical specifications were excluded.

No direct comparison was made amongst MSs with differing *Salmonella* vaccination policies. Only data originating from MS groups with analogous vaccination policies was compared. So MSs were divided into groups with mandatory vaccination, voluntary vaccination or prohibited vaccination. The differentiation between these MS groups was made by an indicator variable in the database. The MSs with mandatory *Salmonella* vaccination were considered to be those for which all holdings were registered in the database as 'vaccination status = yes' (in this case only Germany). The MSs where *Salmonella* vaccination is prohibited were considered to be those for which all holdings were registered in the database as 'vaccination status = no' (the Czech Republic, Denmark, Estonia, Finland, Ireland, Latvia, Luxembourg, Lithuania, Portugal)[1]. The

[1] Vaccination is also prohibited in Sweden, but data about vaccination status was not reported by this Member State.

MSs with voluntary *Salmonella* vaccination were considered to be those for which only a proportion of holdings were registered in the database as 'vaccination status = yes' (Austria, Belgium, Cyprus, Greece, Spain, France, Hungary, Italy, the Netherlands, Poland, Slovenia, the United Kingdom).

Co-linearity between the continuous explanatory variables was verified by the Pearson correlation coefficient. The Pearson chi-square test was used to verify association amongst categorical explanatory variables. For data analysis, a logistic regression model taking account of the degree of within-flock prevalence was developed. The data were analysed at the sample-level including holding as a random effect to control for the expected dependency between samples from the same flock. Main factors were considered in a forward stepwise-selection procedure. Selection of the (relative) most important models was based on Akaike's Information Criterion [1] (twice the negative log likelihood penalized for twice the number of estimated parameters).

Analyses were performed using the statistical software Stata®/SE 9.1 (StataCorp LP, Texas, USA, 2006). The Stata® procedure gllamm "generalized linear and latent mixed models" was used for implementing these cluster-specific random-effects logistic regression models.

[1] Akaike, H. (1974) A New Look at the Statistical Model Identification. IEEE Transactions on Automatic Control AC-19, 716-723.

# 4. Results

An overview of the features of the European laying hen population is given in the Preliminary Report.

## 4.1. Observed prevalence of *Salmonella*

### 4.1.1. Observed *Salmonella* holding prevalence

The observed *Salmonella* prevalence in holdings of laying hens in each MS and at EU level as well as for Norway is presented in Table 2. The observed EU prevalence is weighted by the number of laying hen holdings in each MS.

The comparison between the prevalence figures calculated from the full and the validated datasets indicates that there was no systematic exclusion or inclusion of observations from positive flocks. Although there were some differences in the observed holding prevalence figures for a number of MSs, there was no trend suggesting that the prevalence figures were significantly higher to the full compared to the clean dataset. The reported results are therefore based on the validated dataset.

**_Salmonella_ spp. holding observed prevalence**

The presence of *Salmonella* spp. was detected in 1,486 holdings in the EU. This resulted in a Community weighted observed *Salmonella* spp. holding prevalence of 30.8% (95% CI=29.8-31.8). The observed *Salmonella* spp. holding prevalence in the EU ranged from a minimum of 0% (Luxembourg and Sweden) to a maximum of 79.5% (Portugal). A graphical display showing the 95% CIs of the observed prevalence of *Salmonella* spp.-positive holdings for each MS, at Community level, and for Norway is presented in Figure 1.

**_Salmonella_ Enteritidis / _Salmonella_ Typhimurium holding observed prevalence**

The presence of *S.* Enteritidis / *S.* Typhimurium was detected in 986 holdings in the EU. This resulted in a Community weighted *S.* Enteritidis / *S.* Typhimurium observed holding prevalence of 20.4% (95% CI=19.5-21.3) with a range from 0% (Ireland, Luxembourg, Latvia, and Sweden) to 62.5% (Czech Republic). A graphical display showing the 95% CIs of the observed prevalence of *S.* Enteritidis / *S.* Typhimurium- positive holdings for each MS, at Community level, and for Norway, is presented in Figure 2.

**Observed holding prevalence for _Salmonella_ Enteritidis, for _Salmonella_ Typhimurium and for serovars other than _Salmonella_ Enteritidis and _Salmonella_ Typhimurium**

The observed prevalences for every MS, at EU level, and for Norway, of holdings positive for *S.* Enteritidis, *S.* Typhimurium and for serovars other than *S.* Enteritidis and *S.* Typhimurium are presented in Annex IV. The Community weighted *S.* Enteritidis observed holding prevalence was 18.3% (95% CI=17.5-19.2); for *S.* Typhimurium it was 2.6% (95% CI=2.2-3.0), whereas for serovars other than *S.* Enteritidis and *S.* Typhimurium it was 17.1% (95% CI=16.3-18.0). The latter serovar group contained also the non-typeable serovars. Graphical displays of the 95% CIs

of these prevalences for every MS, at EU level, and for Norway, are shown in Annex V, Annex VI and in Annex VII, respectively.

**Table 2. Observed prevalence of *Salmonella*-positive holdings of laying hens in the EU, 2004-2005**

| | | ***Salmonella* spp.** | | ***Salmonella* Enteritidis and/or Typhimurium** | |
|---|---|---|---|---|---|
| | **N** | **% pos** | **CI 95%[a]** | **% pos** | **CI 95%** |
| Austria | 337 | 15.4 | 12.7 - 18.5 | 10.7 | 8.4 - 13.4 |
| Belgium | 141 | 37.6 | 31.4 - 44.1 | 27.7 | 22.1 - 33.9 |
| Cyprus | 25 | 28.0 | 21.7 - 33.0 | 8.0 | 3.7 - 12.3 |
| Czech Republic | 64 | 65.6 | 61.3 - 68.2 | 62.5 | 58.0 - 65.2 |
| Denmark | 185 | 2.7 | 1.6 - 4.3 | 1.6 | 0.8 - 3.0 |
| Estonia | 11 | 18.2 | -[b] | 9.1 | - |
| Finland | 250 | 0.4 | 0.0 - 1.6 | 0.4 | 0.0 - 1.6 |
| France | 511 | 17.2 | 14.6 - 20.2 | 8.0 | 6.2 - 10.3 |
| Germany | 553 | 28.9 | 25.7 - 32.3 | 24.2 | 21.2 - 27.5 |
| Greece | 140 | 49.3 | 42.8 - 55.5 | 25.7 | 20.5 - 31.6 |
| Hungary | 267 | 43.8 | 39.9 - 47.6 | 33.7 | 30.0 - 37.4 |
| Ireland | 146 | 1.4 | 0.6 - 2.6 | 0.0 | 0.0 - 0.7 |
| Italy | 367 | 29.2 | 25.4 - 33.1 | 7.9 | 5.9 - 10.5 |
| Latvia | 6 | 16.7 | 1.0 - 46.8 | 0.0 | 0.0 - 29.1 |
| Lithuania | 9 | 44.4 | 22.6 - 62.9 | 44.4 | 22.6 - 62.9 |
| Luxembourg | 9 | 0.0 | - | 0.0 | - |
| Poland | 328 | 76.2 | 72.0 - 79.9 | 55.5 | 50.8 - 60.0 |
| Portugal | 44 | 79.5 | 66.7 - 87.7 | 47.7 | 34.9 - 60.4 |
| Slovenia | 98 | 19.4 | 15.4 - 23.8 | 9.2 | 6.4 - 12.7 |
| Spain | 485 | 73.2 | 70.1 - 76.0 | 51.5 | 48.2 - 54.8 |
| Sweden | 168 | 0.0 | 0.0 - 1.3 | 0.0 | 0.0 - 1.3 |
| The Netherlands | 409 | 15.4 | 12.6 - 18.6 | 7.8 | 5.9 - 10.4 |
| The United Kingdom | 454 | 11.9 | 9.9 - 14.7 | 7.9 | 6.2 - 10.1 |
| EU[c] | 5,007 | 29.7 | | 19.7 | |
| **EU weighted prevalence** | | **30.8** | **29.8 - 31.8** | **20.4** | **19.5 - 21.3** |
| Norway | 303 | 0.0 | 0.0 - 0.8 | 0.0 | 0.0 - 0.8 |

[a]: Confidence interval

[b]: No confidence interval for Estonia and Luxembourg since all holdings in these MSs were sampled

[c]: These EU figures do not include data for Malta and Slovakia

**Figure 1. Observed prevalence of *Salmonella*-positive holdings of laying hens, with 95% confidence intervals, in the EU, 2004-2005**

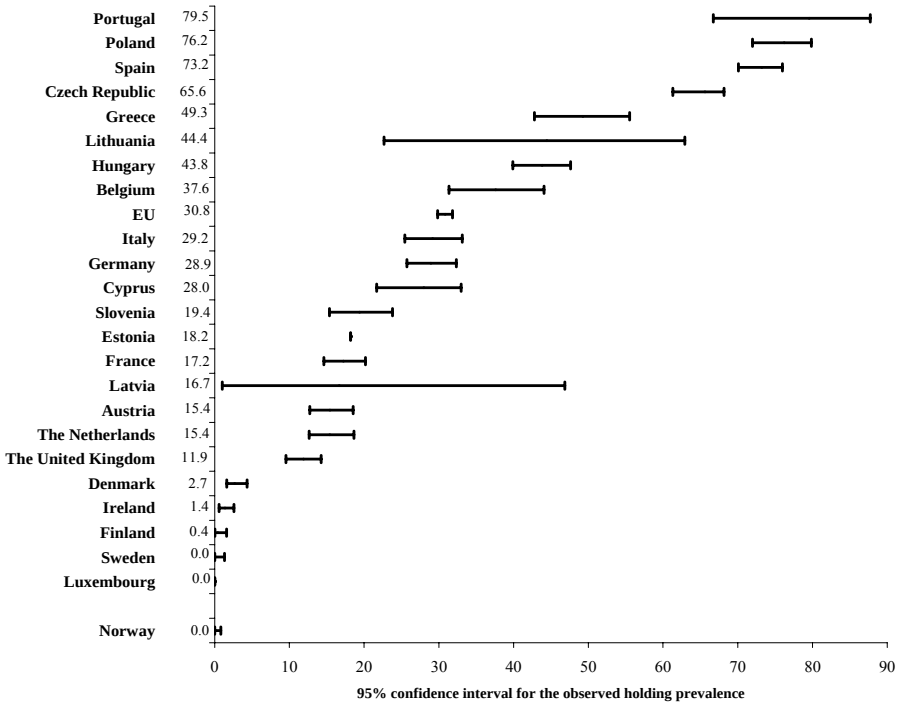
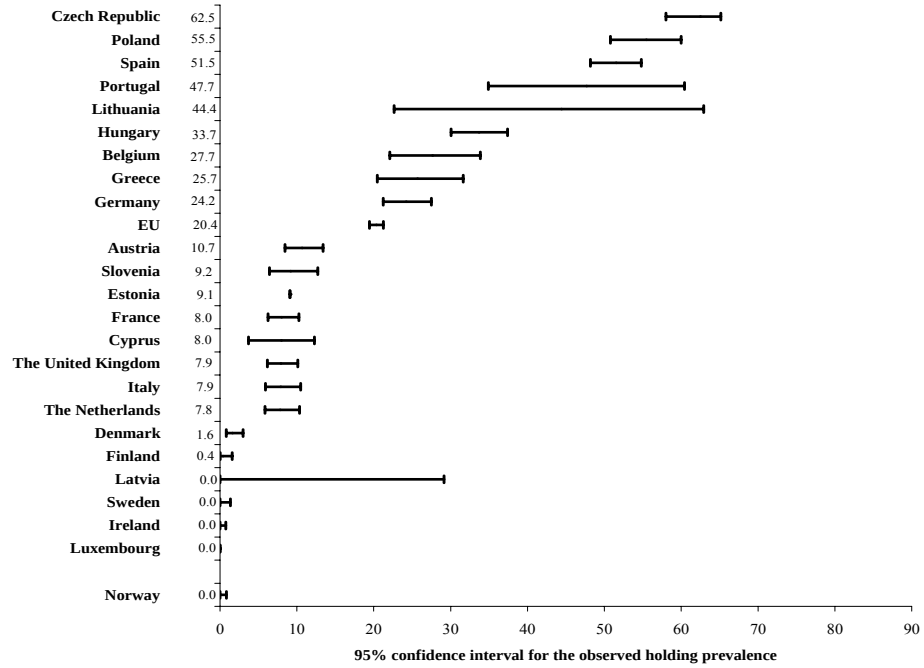



**Figure 2. Observed prevalence of *Salmonella* Enteritidis/Typhimurium-positive holdings of laying hens, with 95% confidence intervals, in the EU, 2004-2005**



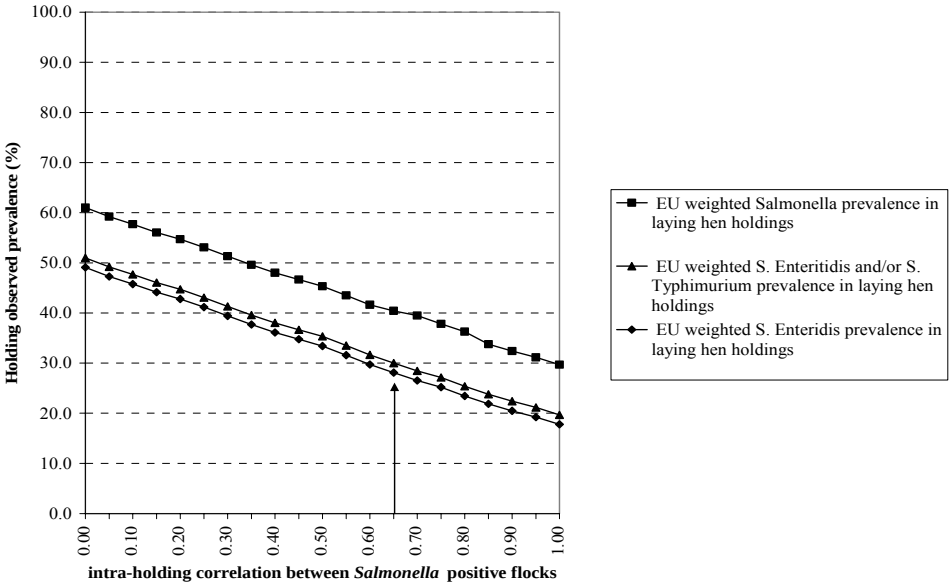
The final Community weighted *Salmonella* observed holding prevalence did not change in a noticeable way compared to the Preliminary Report. This was also the case for almost all MSs. However, the *Salmonella* spp. observed holding prevalence in Greece increased with 11.9%, mostly due an increase in the observed holding prevalence for serovars other than *S.* Enteritidis and *S.* Typhimurium that increased with 12.4%. Cyprus and Lithuania reported decreased *Salmonella* holding observed prevalences, with a respective decrease of 22.0% and 5.6%. Lithuania had the same decrease for its *S.* Enteritidis / *S.* Typhimurium holding observed prevalence, whereas Cyprus reported an increase of 8.0% for this prevalence.

## 4.1.2. Sensitivity analysis with respect to the study design

### 4.1.2.1 Design-bias

The results of the simulation exploring the impact of the bias in the study design are presented in Figure 3. In this figure the horizontal axis represents the *Salmonella* intra-holding correlation coefficient between flocks in a holding. These results demonstrate that if there is not a 100% correlation between flocks in a holding, that is, if they are not all *Salmonella*-positive or all *Salmonella*-negative (meaning that the *Salmonella* intra-holding correlation coefficient is lower than 1), the observed prevalence of *Salmonella*-positive holdings would be higher than the actual reported prevalence in the study. For example, if the *S.* Enteritidis intra-holding correlation coefficient is assumed to be 0.65 (see mark in Figure 3), which is an estimate for Northern Ireland published in 2001 by S. McDowell[1], the observed prevalence of *S.* Enteritidis-positive holdings in the Community would be 28% instead of 18% as currently reported. This discrepancy results from the chance of randomly sampling a negative flock on holdings where other positive flocks may be present.

**Figure 3. The effect of the intra-holding correlation on the *Salmonella* prevalence in laying hen holdings**




[1] http://www.rivm.nl/crlsalmonella/workshop/WorkshopVIII/McDowell%20150503/sld001.htm

#### 4.1.2.2 Simulation of the observed prevalence of *Salmonella* Enteritidis-positive holdings as a function of number and type of samples taken in the holding

The reduced set of comparable data used for the simulation of the EU observed prevalence of *S.* Enteritidis-positive holdings, contained 4,730 holdings and 33,110 samples. All these holdings had five faeces and two dust samples analysed, in compliance with the technical specifications, and were of known flock production type and known vaccination status.

The simulation results are presented in Figure 4. The simulated EU observed prevalence of *S.* Enteritidis-positive holdings is displayed for all combinations of dust samples (0 or 1 or 2) for a given number of faeces samples. For the scenario of seven samples taken (five faeces and two dust) the median simulated estimate of EU observed prevalence of *S.* Enteritidis-positive holdings was 16.8%, and the 95% uncertainty interval ranged from 15.8% to 17.8%. Although comparable to the estimated EU observed prevalence of *S.* Enteritidis-positive holdings of 18.3% (95% CI 17.5-19.2) (Annex IV) the simulated prevalence was lower. This is explained by the difference in datasets on which the analyses were based.

The simulation demonstrates that a reduced number of samples would lead to an estimate of EU observed prevalence of *S.* Enteritidis-positive holdings that is lower than that observed with a sample size of seven. This decrease in observed prevalence appears to be marginal when the number of samples is reduced to six, five or four. With fewer than four samples the observed prevalence would be significantly lower. If only one sample is taken - either one faeces or one dust sample - the estimate of EU observed prevalence of *S.* Enteritidis-positive holdings would be reduced to a median of 8.3%, which is approximately half the median estimate of 16.8% when seven samples are taken. The number of samples taken therefore has an influence on the accuracy of the prevalence estimate.

The sample type has also a clear impact on prevalence estimates. Reducing the number of dust samples will result in a larger reduction in the prevalence estimate. However, this observation is invalid if only one or two samples are taken. With only two samples, the most sensitive detection is with one faeces and one dust sample. If one sample is taken, the two options - either one faeces or one dust sample - appear to be equally sensitive.

### 4.1.3. *Salmonella* within-flock proportion positive samples

A total of seven samples was taken from each sampled laying hen house, and in positive holdings one to seven samples could have been positive. The number of samples positive for *S.* Enteritidis / *S.* Typhimurium amongst the positive flocks varied between one and seven, but an important proportion (44%) of the *S.* Enteritidis / *S.* Typhimurium positive holdings was found positive on the basis of only one or two positive samples. The distribution of the within-flock number of *S.* Enteritidis / *S.* Typhimurium-positive samples in the positive flocks in each MS is shown in Figure 5. This distribution varied amongst the MSs. Some, such as France, Germany, Greece and Italy, had a smaller number of positive samples per flock. Others, such as Belgium and the United Kingdom had a greater number of positive samples per flock. The figures show that there are different distributions of positive samples in the MSs. The number of samples positive for *Salmonella* varied between one and seven and 38% of the *Salmonella* positive holdings was found positive on the basis of only one or two *Salmonella* positive samples.

**Figure 4. Simulated EU-weighted prevalence of *Salmonella* Enteritidis-positive laying hen holdings and 95% uncertainty intervals for varying sample sizes and types**

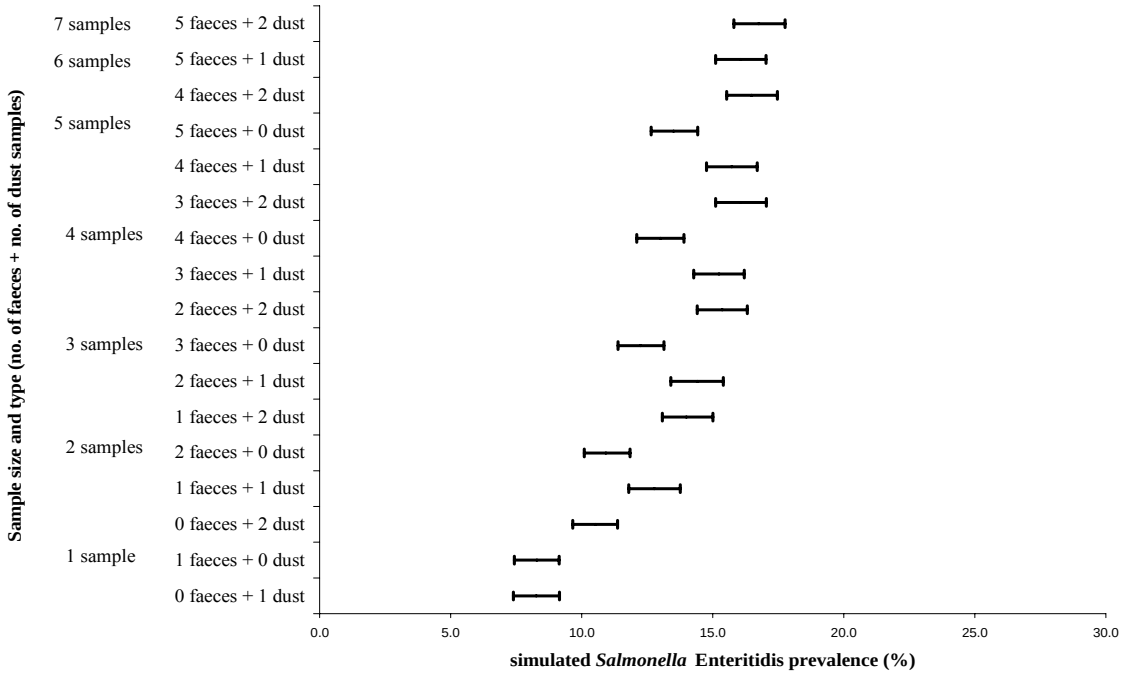
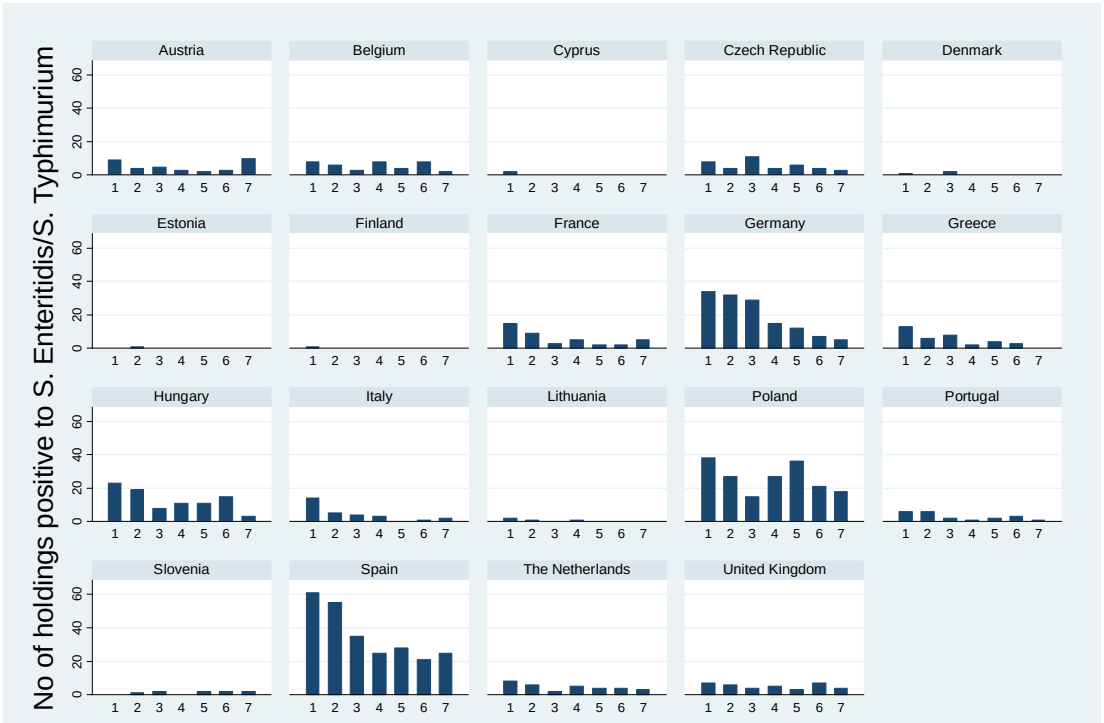



**Figure 5. Distribution of the within-flock number of *Salmonella* Enteritidis/Typhimurium positive samples in positive flocks observed in the EU MSs, 2004-2005**



## 4.2. Frequency distribution of *Salmonella* serovars

The serotyping of *Salmonella* was mandatory according to the technical specifications of the study. At least one isolate from each positive sample was to be typed according to the Kaufmann-White Scheme. Results from any holding where the serovar information was not available for any isolate were excluded from the full dataset. There were 5,668 (16.2% of 35,049) *Salmonella*-positive samples originating from 1,486 positive holdings. Two different *Salmonella* serovars were isolated from 30 *Salmonella*-positive samples.

The twenty most frequently isolated *Salmonella* serovars in the EU are listed in Table 3. This table is ranked based on the percentages of specific *Salmonella* serovar-positive holdings, as this is the epidemiological unit of interest. Member State-specific overviews of the most frequently isolated serovars are shown in Annex VIII. Luxembourg, Sweden and Norway did not detect any *Salmonella* positive sample. The serovar frequency distribution for the EU as well as for each MS was based on the number of typed isolates, including non-typeable isolates.

**Table 3. Frequency distribution of isolated *Salmonella* serovars in the laying hens baseline study, 2004-2005**

| Serovars (N=5,698) | | | No of Member States | Holdings with serovars (N=1,486) | |
|---|---|---|---|---|---|
| | N | % | reporting the serovar | N | % |
| S. Enteritidis | 2,980 | 52.3 | 18 | 890 | 59.9 |
| S. Infantis | 481 | 8.4 | 13 | 171 | 11.5 |
| S. Typhimurium | 273 | 4.8 | 15 | 123 | 8.3 |
| S. Mbandaka | 231 | 4.1 | 12 | 98 | 6.6 |
| S. Livingstone | 155 | 2.7 | 10 | 50 | 3.4 |
| S. Hadar | 118 | 2.1 | 7 | 50 | 3.4 |
| S. subsp. enterica rough | 101 | 1.8 | 2 | 50 | 3.4 |
| S. Virchow | 140 | 2.5 | 8 | 40 | 2.7 |
| S. Ohio | 103 | 1.8 | 2 | 35 | 2.4 |
| S. Agona | 64 | 1.1 | 12 | 32 | 2.2 |
| S. Braenderup | 78 | 1.4 | 8 | 29 | 2.0 |
| S. Tennessee | 52 | 0.9 | 9 | 28 | 1.9 |
| S. Montevideo | 74 | 1.3 | 9 | 26 | 1.7 |
| S. Bredeney | 63 | 1.1 | 5 | 26 | 1.7 |
| S. Senftenberg | 35 | 0.6 | 9 | 25 | 1.7 |
| S. Anatum | 31 | 0.5 | 4 | 21 | 1.4 |
| S. Rissen | 29 | 0.5 | 8 | 17 | 1.1 |
| S. Indiana | 28 | 0.5 | 4 | 11 | 0.7 |
| S. Newport | 33 | 0.6 | 7 | 10 | 0.7 |
| S. Altona | 26 | 0.5 | 2 | 10 | 0.7 |
| Other serovars | 528 | 9.3 | | | |
| *S.* non typeable | 75 | 1.3 | 8 | 36 | 2.4 |

*S.* Enteritidis was by far the most common serovar in laying hen flocks in the survey. It was found in samples from 18 MSs and in 59.9% (890 out of 1,486) *Salmonella*-positive holdings. *S.* Infantis was isolated in samples from 13 MSs and in 11.5% (171 out of 1,486) *Salmonella*-positive holdings. The third and fourth most frequently isolated serovars at holding level were *S.* Typhimurium and *S.* Mbandaka which were found in samples from 15 and 12 MSs respectively.

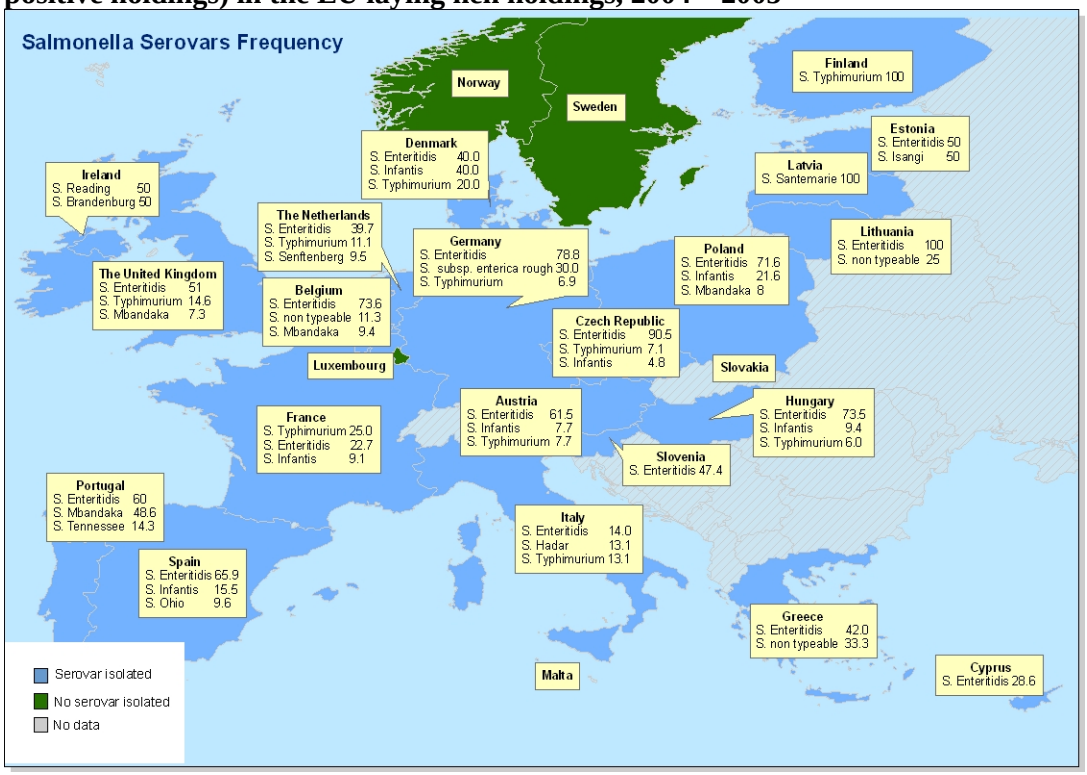
The percentage *S.* Enteritidis-positive holdings in relation to the total number of *Salmonella*-positive holdings ranged from 14.0% (Italy) to 90.5% (Czech Republic) in the MSs. In all MSs that isolated *S.* Enteritidis, this serovar was by far the most isolated in positive holdings.

Exceptions to this observation were Denmark and Estonia where, respectively, *S*. Infantis and *S*. Isangi were isolated at the holding-level as frequently as *S.* Enteritidis, and France where *S.* Enteritidis was only the second most isolated in positive holdings, with *S*. Typhimurium being the first one. Some MSs did not identify any *S.* Enteritidis (Finland, Ireland and Latvia), while Lithuania only isolated *S.* Enteritidis but had one non-typeable isolate.

The frequency of *S*. Infantis isolates ranged from 3.8% (Belgium) to 21.6% (Poland). The MSs that did not identify any *S*. Infantis were Cyprus, Estonia, Finland, Ireland, Latvia, Lithuania, Portugal and the Netherlands, although Cyprus and Portugal had some non-typeable serovars. The *S.* Typhimurium frequency in the MSs varied from 2.8% (Poland) to 25.0% (France) in the MSs reporting the serovar. Finland had one positive holding and *S.* Typhimurium was isolated (100.0%). The MSs that did not isolate any *S.* Typhimurium amongst the typeable serovars were Cyprus, Estonia, Ireland, Latvia, Lithuania, and Slovenia. The frequency of *S.* Mbandaka ranged from 1.4% (Greece) to 48.6% (Portugal). Figure 6 displays for the MSs the most frequently isolated *Salmonella* serovars. No clear geographical distribution pattern is apparent in the figure.

In addition to the above mentioned observations, Figure 6 shows that in Hungary, Poland and Spain, *S*. Infantis was the second highest percentage. Also in Austria *S*. Infantis was isolated in the second highest number of positive holdings, together with *S.* Typhimurium. *S.* Typhimurium was isolated in the second highest number of positive holdings in Czech Republic, The Netherlands, The United Kingdom, and Italy (together with *S.* Hadar). Belgium, Greece and Lithuania have reported 'non typeable serovars' in the second highest number of positive holdings.

**Figure 6. Most frequently identified *Salmonella* serovars (the percentage of the *Salmonella* positive holdings) in the EU laying hen holdings, 2004 – 2005**




In the case that the second or third highest percentage positive holdings were the same for more than one serovar, these percentages were not displayed.

## 4.3. Analysis of factors associated with *Salmonella* positivity

The dataset of strictly comparable data used for the analysis of factors associated with the occurrence of *Salmonella* contained 3,808 holdings and 26,656 samples. All these holdings had five faeces and two dust samples in compliance with the technical specifications and were of known flock production type and known vaccination status[1].

The recorded number of hens in the holding present at sampling was correlated with the number of flocks in the holding, and with the number of hens in the flock present at sampling. The significant correlation coefficients between these variables were at least 0.65. The holding and flock size variables were all associated with the flock production type. Almost two-thirds of the holdings in the study were of the cage production type, and most of these belonged to the largest size categories. Organic[2] flocks were on average of the smallest size, whereas the barn and the free-range standard flocks were of low to medium size. Based on this observation, it was decided to take account only of flock production type in the regression models, bearing in mind that the effect of the production type and size parameters cannot be disentangled in the analyses. Thus the findings related to flock production type apply also to the holding and flock size variables. The risk factor analyses were performed separately for *S.* Enteritidis, *S.* Typhimurium and for serovars other than *Salmonella* Enteritidis and Typhimurium.

### 4.3.1. EU and Member States -group analyses of factors associated with *Salmonella* positivity

#### 4.3.1.1 *Salmonella* Enteritidis

As well as analysing the results for the EU as a whole, two MS groups were investigated. The groups were based on point estimates of their observed prevalence of *S.* Enteritidis-positive holdings:

1. MSs having an observed prevalence of *S.* Enteritidis-positive holdings between 2.5% and 15% (intermediate prevalence MS group),
2. MSs having an observed prevalence of *S.* Enteritidis-positive holdings above 15% (highest prevalence MS group).

Due to the very low number of *S.* Enteritidis-positive holdings, no regression analysis was carried out for the MS group with an observed prevalence of *S.* Enteritidis-positive holdings below 2.5% (lowest prevalence MS group).

A general overview of the factors associated with finding *S.* Enteritidis on sampled holdings, as well as their relative importance, is shown in Table 4. The table shows that the factors that were not statistically significantly associated with the occurrence of *S.* Enteritidis on the holdings, either at EU level or in either of the MS groups were;

- the flock age type (mixed ages or homogenous age birds),
- medication of the flock with antimicrobials within two weeks prior to sampling

[1] The vaccines referred to in this baseline study covered all vaccines against *Salmonella* serovars.

[2] An organic flock system is a production type that is similar to the free-range system; however there are additional guidelines for feed and veterinary requirements. Also the pullets should be raised by certified organic production methods from hatch and poultry must have access to outdoors whenever the weather permits and for at least a third of their life.

- month of the sampling (seasonality)
- time gap (in days) between date of sampling and date of start of bacteriological detection testing in the laboratory

A detailed description of the factors and how they relate to *S.* Enteritidis positivity can be found in Annex IX.

**Table 4. Ranking of factors associated with the occurrence of *S.* Enteritidis in the laying hen holdings in the EU and in MS-groups, 2004-2005**

| Factor | EU | MS-group with observed *S.* Enteritidis holding prevalence | |
|---|---|---|---|
| | | *between 2.5% - 15%* | *above 15%* |
| the sample type of (dust or faeces) | 3[a] | 2 | 2 |
| the flock production type | 1 | 1 | 4 |
| the age of the hens in the flock | 4 | - | 3 |
| the flock age type | -[b] | - | - |
| medication status | - | - | - |
| vaccination status | 2 | - | 1 |
| month of sampling | - | - | - |
| days to bacteriological testing | - | - | - |

[a]: the factor was associated with *S.* Enteritidis prevalence, '1' being the most associated one

[b]: the factor was not associated with *S.* Enteritidis positivity, given the association of the other factors

The results of the statistical analysis of factors associated with *S.* Enteritidis positivity at the EU level are detailed in Table 5**Error! Reference source not found.** in a descending order of importance. If the P-value is smaller than 0.05, the difference in *S.* Enteritidis positivity to the compared basis is considered significant. In such cases the odds ratio (OR) differs significantly from one.

**Table 5. Factors associated with the occurrence of *S.* Enteritidis in the laying hen holdings in the EU, 2004-2005**

| Factor | OR[1] | CI 95% | P-value |
|---|---|---|---|
| Flock production type (basis for comparison: cages) | | | |
| barn | 0.57 | [0.34 0.97] | 0.040 |
| free-range standard | 0.02 | [0.01 0.04] | < 0.001 |
| organic | 0.05 | [0.02 0.14] | < 0.001 |
| Vaccination status (basis for comparison: unvaccinated status) | | | |
| SE[2]-vaccinated | 0.12 | [0.07 0.20] | < 0.001 |
| Vaccinated with non-SE | 0.46 | [0.25 0.83] | 0.010 |
| Sample type: dust (basis for comparison: faeces) | 1.54 | [1.35 1.76] | < 0.001 |
| Age of the hens in the flock (weeks) | 1.02 | [1.01 1.03] | < 0.001 |

OR[1]: odds ratio controlling for the MSs' vaccination policy
SE[2]: *S.* Enteritidis

The MS-group with an observed *S.* Enteritidis holding prevalence above 15% consisted of Belgium, Czech Republic, Germany, Greece, Hungary, Lithuania, Poland, Portugal and Spain. This MS-group contained overall 1,692 holdings and 11,844 samples. The factors associated with the occurrence of *S.* Enteritidis in this group are shown in Table 6, a descending order of importance. The factors associated with *S.* Enteritidis occurrence are the same as at the EU level but with a different order of importance. For this highest prevalence MS group, the factor with the greatest influence on risk is the vaccination status of the flocks, whereas the flock production type is only the 4th most important factor.

**Table 6. Factors associated with the occurrence of *S.* Enteritidis in the laying hen holdings in MSs having an observed S. Enteritidis holding prevalence above 15%, 2004-2005**

| Factors | OR[1] | CI 95% | P-value |
|---|---|---|---|
| Vaccination status (basis for comparison: unvaccinated status) | | | |
| SE[2]-vaccinated | 0.21 | [0.13 0.34] | < 0.001 |
| Vaccinated with non-SE vaccine | 0.47 | [0.27 0.80] | 0.005 |
| Sample type: dust (basis for comparison: faeces) | 1.47 | [1.27 1.69] | < 0.001 |
| Age of the hens in the flock (weeks) | 1.01 | [1.00 1.02] | 0.010 |
| Flock production type (basis for comparison: cages) | | | |
| barn | 1.37 | [0.83 2.23] | 0.215 |
| free-range standard | 0.30 | [0.10 0.90] | 0.032 |
| organic | 2.00 | [0.59 6.76] | 0.266 |

OR[1]: odds ratio controlling for the MSs' vaccination policy
SE[2]: *S.* Enteritidis

The MS group with an observed prevalence of *S.* Enteritidis-positive holdings between 2.5 and 15% consisted of Austria, Cyprus, Estonia, France, Italy, Slovenia, the Netherlands and the United Kingdom. Altogether, the group provided 1,533 holdings and 10,731 samples. The factors associated with occurrence of *S.* Enteritidis are presented in Table 7 in a descending order of importance. Compared to the EU level, fewer factors had an impact on the result but flock type, the most important factor, was the same as for the EU level. Unlike the EU level and the higher prevalence MS group, vaccination status was not significantly associated with the risk of *S.* Enteritidis in this intermediate prevalence MS group.

**Table 7. Factors associated with the occurrence of *S.* Enteritidis in the laying hen holdings in MSs having an observed *S.* Enteritidis holding prevalence between 2.5 and 15%, 2004-2005**

| Factor | OR[1] | CI 95% | P-value |
|---|---|---|---|
| Flock production type (basis for comparison: cages) | | | |
| barn | 0.14 | [0.02 1.11] | 0.063 |
| free-range standard | 0.06 | [0.01 0.42] | 0.004 |
| organic | 0.05 | [0.00 0.61] | 0.019 |
| Sample type: dust (basis for comparison: faeces) | 2.28 | [1.50 3.46] | < 0.001 |

OR[1]: odds ratio controlling for the MSs' vaccination policy

At the EU level, cage production was associated with a increased risk of *S.* Enteritidis positivity compared to barn, organic, and free-range standard production. Barn production was associated with a higher risk than organic and free-range standard production, which had a similar low risk. Cage production was also a significant risk factor in the two MS-groups analysed. However, in the high prevalence MS group, the risk of *S.* Enteritidis positivity was significantly higher only when compared to free-range standard production. In the intermediate prevalence MS group, the risk of *S.* Enteritidis positivity was significantly lower both in free-range standard and organic production, but not in barn production, when compared to cage production.

The distribution of *S.* Enteritidis-positive holdings in different flock production types is illustrated in Figure 7. In this figure the observed *S.* Enteritidis holding prevalence is presented in a simplified way in which the impact of other important variables, such as the number of hens and flocks on a holding, is not taken into account. Still, the patterns found, clearly support the associations found in the statistical analyses. The distribution of *S.* Enteritidis-positive holdings according to the number of hens on the holding, which was a variable that was associated with the flock production type, is presented in Figure 8.

At the global EU level, vaccination of flocks with either *S.* Enteritidis or non-*S.* Enteritidis type of vaccines was found to decrease the risk of *S.* Enteritidis positivity when compared to unvaccinated flocks. This was also the case for the MSs group having the highest prevalence, whereas vaccination was not associated with *S.* Enteritidis positivity in the intermediate prevalence MS group. The observed *S.* Enteritidis prevalence by vaccination status is presented in Figure 9. Even though differences between vaccination status are not that clear, the multiple regression analysis indicates that vaccination protects against *S.* Enteritidis infection particularly in the high prevalence MSs group.

**Figure 7. Observed *S*. Enteritidis holding prevalence by flock production type in EU, 2004-2005**

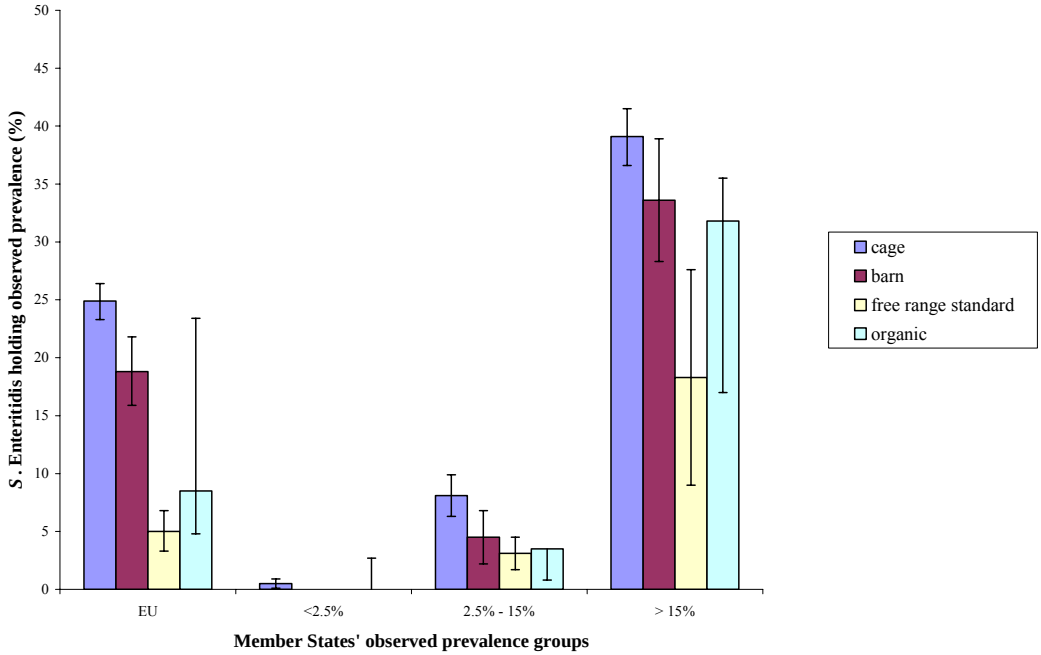
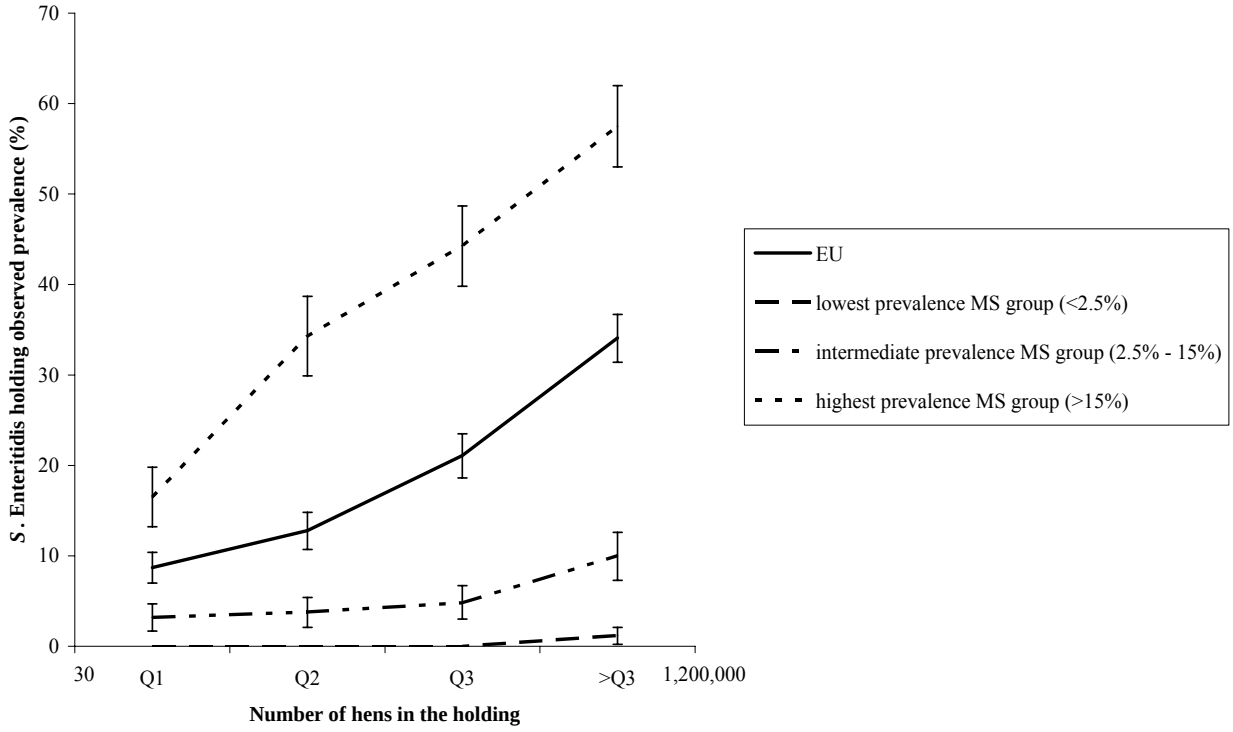
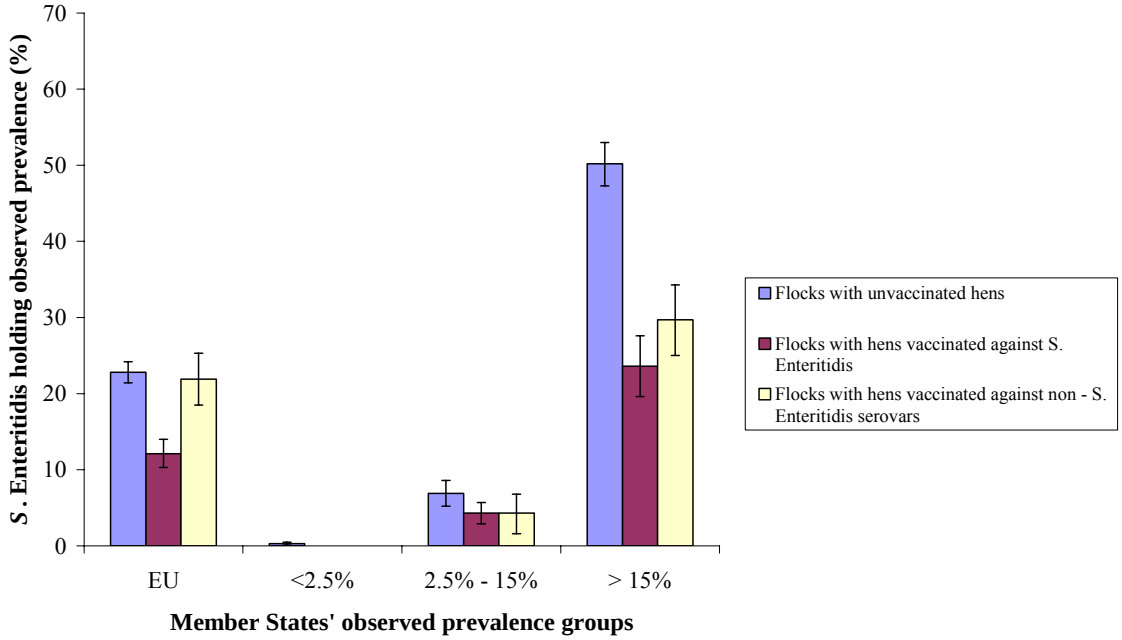



**Figure 8. Observed *S*. Enteritidis holding prevalence by number of hens in the holding in EU, 2004-2005**




Q1, Q2, and Q3: Quartiles 1 to 3 (The 'Quartiles 1 to 3' are the lower, middle and upper quartiles and they divide the data in categories with, respectively, all values being smaller than 25%, 50% and 75% of the remaining data, e.g. see data values for the EU in Annex IX quartile 1= 3,000; quartile 2= 8,000 and quartile 3 = 23,000).

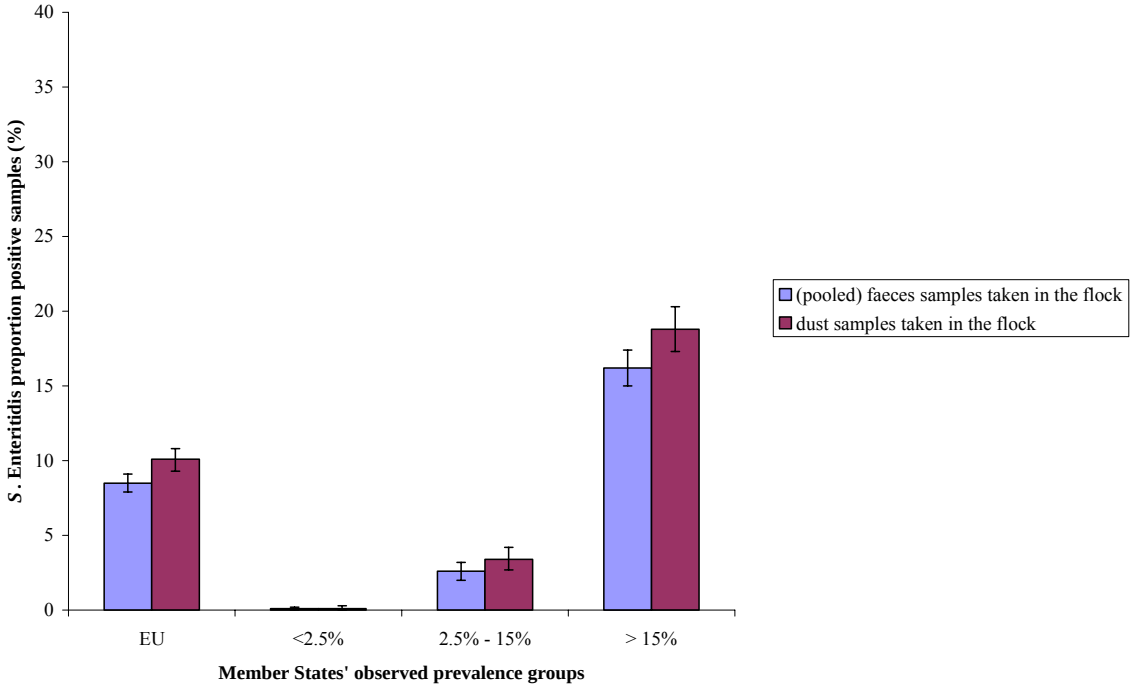
**Figure 9. Observed *S*. Enteritidis holding prevalence by flock vaccination status in EU, 2004-2005**




Samples of dust were more likely to be positive for *S.* Enteritidis compared with faeces samples (Figure 10), which was also an outcome of the statistical analysis (Tables 5-7) in all groups and at the EU-level.

An increase in the age of the hens present in the flock at sampling was related to a higher observed prevalence of *S.* Enteritidis at both the EU level and in the MS group with the highest prevalence.

**Figure 10. Observed *S*. Enteritidis sample prevalence by sample type in EU, 2004-2005**



#### 4.3.1.2 *Salmonella* Typhimurium

The factors associated with the occurrence of *S.* Typhimurium on holdings were investigated at both the EU level and for the MS group having at least one holding with *S*. Typhimurium. The MS group consisted of Austria, Belgium, the Czech Republic, Germany, Denmark, Greece, Spain, Finland, France, Hungary, Italy, the Netherlands, Poland, Portugal and the United Kingdom. This group provided 3,538 holdings and 24,766 samples. The results of the analysis of these factors were analogous at both EU and at MS group level.

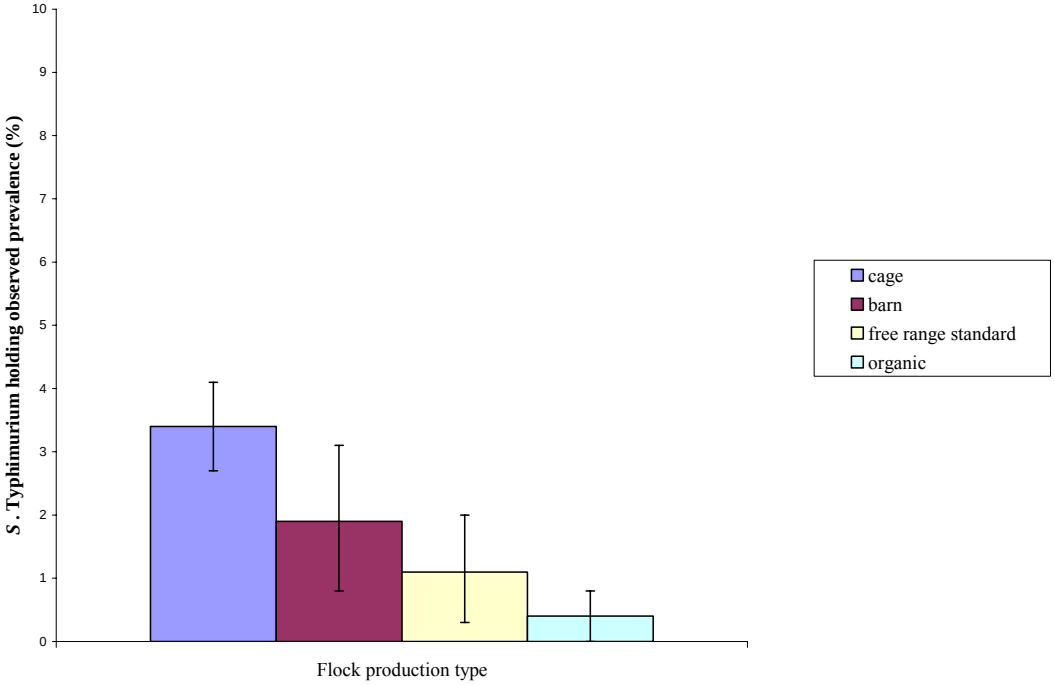
The only two factors associated with *S.* Typhimurium positivity were flock production type (cage, barn, free-range standard or organic) and type of sample taken (dust or faeces samples) (Table 8). A detailed description of the factors and how they relate to *S.* Typhimurium positivity can be found in Annex IX.

**Table 8. Factors associated with the occurrence of *S*. Typhimurium in the laying hen holdings in MSs having *S*. Typhimurium positive holdings, 2004-2005**

| Factor | OR[1] | CI 95% | P-value |
|---|---|---|---|
| Flock production type (basis for comparison: cages) | | | |
| barn | 0.23 | [0.07 0.81] | 0.022 |
| free-range standard | 0.07 | [0.02 0.31] | < 0.001 |
| organic | 0.07 | [0.01 0.63] | 0.018 |
| Sample type: dust (basis for comparison: faeces) | 1.76 | [1.20 2.58] | 0.004 |

OR[1]: odds ratio controlling for the MSs' vaccination policy

**Figure 11. Observed *S*. Typhimurium holding prevalence by flock production type, for MSs having *S*. Typhimurium positive holdings, 2004-2005**



The observed prevalence of *S.* Typhimurium was the highest in cage production systems. The prevalence was significantly lower in barn, free-range standard and organic flock types compared to cage flocks. Samples of dust were significantly more likely to be positive for *S.* Typhimurium than faeces samples.

The distribution of *S.* Typhimurium observed holding prevalence by flock production type is presented in Figure 11.

#### 4.3.1.3 Serovars other than *Salmonella* Enteritidis and *Salmonella* Typhimurium

Factors significantly associated with the occurrence of serovars other than *S.* Enteritidis and *S.* Typhimurium were investigated both at the EU level, as well as for the MS group with at least one holding where one of these serovars was found. The MS group consisted of Austria, Belgium, Cyprus, the Czech Republic, Germany, Denmark, Estonia, Greece, Spain, Finland, France, Hungary, Ireland, Italy, Lithuania, Latvia, the Netherlands, Poland, Portugal, Slovenia and the United Kingdom. It contained 3,552 holdings and 24,864 samples. The results were analogous for the EU level and for the MS group, with the exception of one additional risk factor for the MS group. It was therefore decided to present only the results of analysis for the MS group.

The factors associated positivity to serovars other than *S.* Enteritidis and *S.* Typhimurium were, in decreasing order of importance (Table 9):

- flock production type (cage, barn, free-range standard or organic),
- type of sample taken (dust or faeces samples),
- age (in weeks) of the hens present in the flock at sampling,
- month of sampling,
- flock vaccination status (unvaccinated or *Salmonella*-vaccinated), and
- time in days between date of sampling and date of start of bacteriological detection testing in the laboratory.

It is noteworthy, that more factors were associated with holdings being positive for other serovars than *S.* Enteritidis and *S.* Typhimurium than with holdings being positive for *S.* Enteritidis or *S.* Typhimurium.

For the other serovars, the cage production was associated with a higher risk for positivity compared to the other flock production types. Samples of dust were significantly more likely to be positive for serovars other than *S.* Enteritidis and *S.* Typhimurium compared to faeces samples. Increasing age of the hens in the sampled flock was also related to a higher observed prevalence. Holdings where flocks were vaccinated against any *Salmonella* serovar were less likely to be positive compared to unvaccinated flocks.

The statistical analysis also indicated that the presence of serovars other than *S.* Enteritidis and *S.* Typhimurium was related to the month of the sampling, which is supported by the descriptive analysis in Figure 12. In samples taken during the months of February, September, October and November, the prevalence of serovars other than *S.* Enteritidis and *S.* Typhimurium was higher compared to January.

Finally, as illustrated in Figure 13, an increasing time gap between sampling and the start of bacteriological testing in the laboratory was a significant factor. This factor was not significant at the EU level where more negative samples were included in the analysis. Interestingly, a three-

day delay appeared to give better detection than a 0-2 day delay, but this may be a systematic artefact which reflects laboratory procedures in MS with different prevalences.

**Table 9. Factors associated with the occurrence of serovars other than *S.* Enteritidis and *S.* Typhimurium in the laying hen holdings in MSs having positive holdings, 2004-2005**

| Factor | OR[1] | CI 95% | P-value |
|---|---|---|---|
| Flock production type (basis for comparison: cages) | | | |
| barn | 0.04 | [0.02 0.07] | < 0.001 |
| free-range standard | 0.01 | [0.00 0.02] | < 0.001 |
| organic | 0.02 | [0.01 0.05] | < 0.001 |
| Sample type: dust (basis for comparison: faeces) | 2.54 | [2.19 2.93] | < 0.001 |
| Age of the hens in the flock (weeks) | 1.05 | [1.04 1.06] | < 0.001 |
| Sampling month (basis for comparison: January) | | | |
| February | 2.84 | [1.08 7.43] | 0.034 |
| March | 1.56 | [0.62 3.91] | 0.345 |
| April | 1.16 | [0.47 2.87] | 0.750 |
| May | 1.60 | [0.64 4.02] | 0.317 |
| June | 1.65 | [0.65 4.15] | 0.292 |
| July | 1.00 | [0.38 2.64] | 0.997 |
| August | 2.03 | [0.83 4.97] | 0.121 |
| September | 3.14 | [1.31 7.49] | 0.010 |
| October | 10.71 | [3.65 31.37] | < 0.001 |
| November | 4.96 | [1.82 13.54] | 0.002 |
| December | 1.41 | [0.44 4.49] | 0.563 |
| Vaccination status (basis for comparison: unvaccinated status) | | | |
| Vaccinated against *Salmonella* | 0.50 | [0.33 0.77] | < 0.001 |
| Time gap between sampling and testing (days) | 1.13 | [1.01 1.27] | 0.040 |

OR[1]: odds ratio controlling for the MSs' vaccination policy

**Figure 12. Observed holding prevalence for serovars other than *S*. Enteritidis and *S*. Typhimurium, by month of sampling, for MSs having positive holdings, 2004-2005**

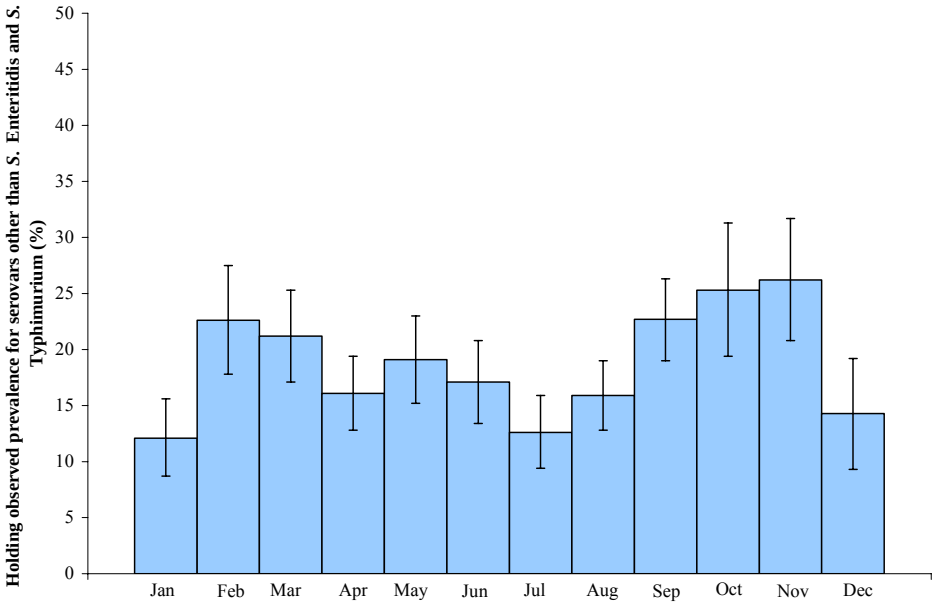
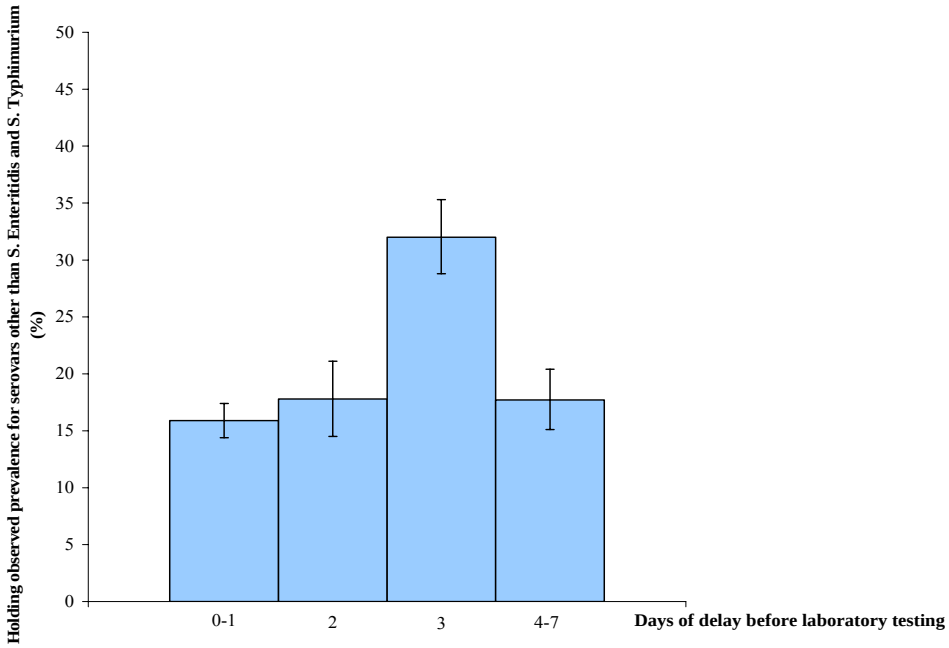



**Figure 13. Observed holding prevalence for serovars other than *S*. Enteritidis and *S*. Typhimurium, by days of delay before laboratory testing was started, for MSs having positive holdings, 2004-2005**



## 4.4. Analysis of additional information reported on a voluntary basis

### 4.4.1. Phage typing

MSs could submit additional information on *S.* Enteritidis and *S.* Typhimurium phage types and antimicrobial susceptibility of *Salmonella* isolates. The study protocol recommended phage typing of at least one isolate of *S.* Enteritidis and *S.* Typhimurium from each positive holding, using the phage typing protocol defined by the Health Protection Agency Colindale, London. The phage typing and antimicrobial susceptibility testing information was not representative of the Community as a whole because of incomplete reporting.

The following results section describes the submitted phage type information. Ranking of *S.* Enteritidis and *S.* Typhimurium phage types was done by adding up the number of each *S.* Enteritidis and *S.* Typhimurium phage type across all the MSs who reported on phage types. The phage type distribution was based on the number of phage typed isolates, including the non-typeable ones.

#### 4.4.1.1 *S.* Enteritidis phage types

Data on *S.* Enteritidis phage types was provided by eight MSs (Austria, the Czech Republic, Denmark, Germany, Italy, Lithuania, the Netherlands and the United Kingdom). Ten MSs with *S.* Enteritidis isolates did not report *S.* Enteritidis phage typing information.

The MSs that gave information on *S.* Enteritidis phage types reported a total of 907 *S.* Enteritidis isolates, out of which 790 isolates were phage typed (92.4%) (Table 10). This represented only 29.0% of the total 3,129 *S.* Enteritidis isolates at EU level. Most frequently reported phage types are presented in Table 10, which also displays the number of MSs and holdings where *S.* Enteritidis phage types were detected. In this table the ranking is based on the percentages of specific *S.* Enteritidis phage type-positive holdings in the EU. MS-specific overviews of *S.* Enteritidis phage types are shown in Annex X.

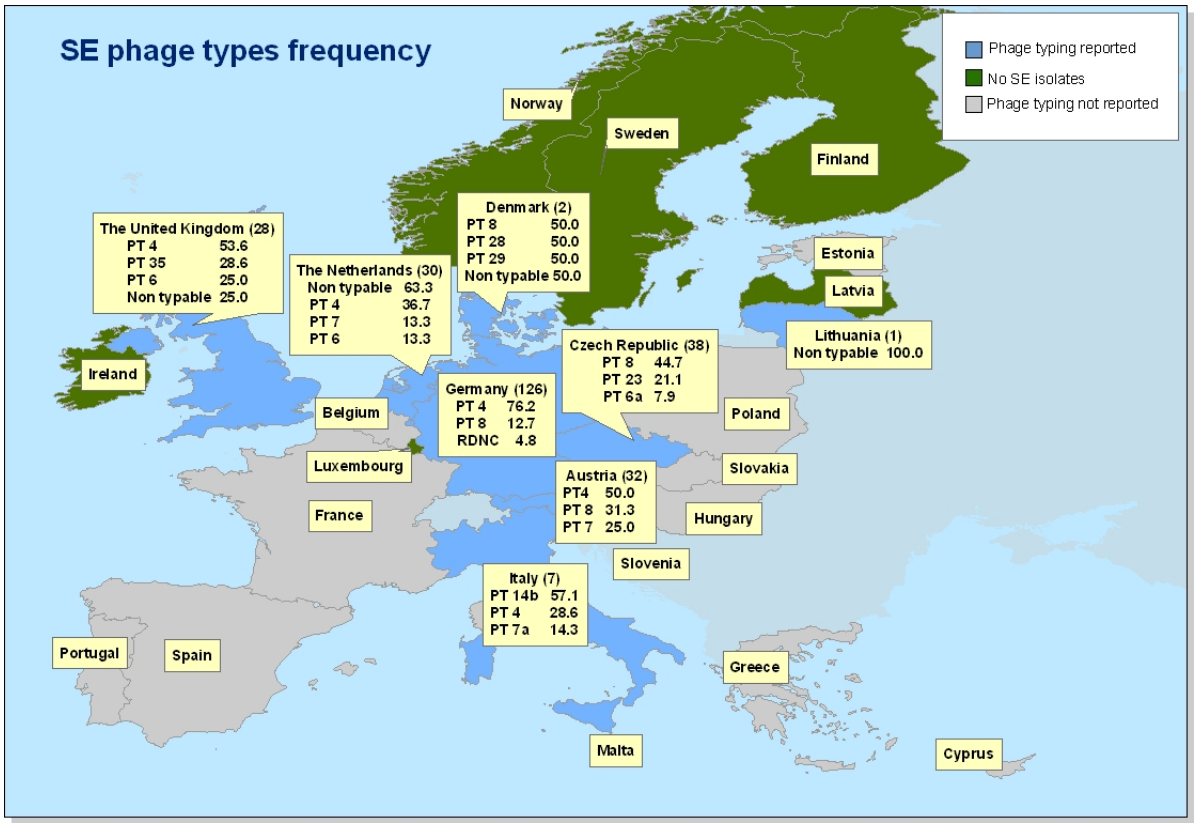
**Table 10. Distribution of *S.* Enteritidis phage types in laying hens in the EU, 2004-2005**

| *S.* Enteritidis (N=790) | | | No of Member States | No of holdings where |
|---|---|---|---|---|
| phage type | N | % | reporting the phage type | the phage type was detected |
| PT 4 | 354 | 44.8 | 5 | 140 |
| PT 8 | 116 | 14.7 | 5 | 47 |
| PT 7 | 51 | 6.5 | 5 | 21 |
| PT 6 | 28 | 3.5 | 5 | 15 |
| PT 23 | 14 | 1.8 | 3 | 12 |
| PT 35 | 26 | 3.3 | 2 | 10 |
| PT 1 | 25 | 3.2 | 5 | 10 |
| PT 21 | 31 | 3.9 | 3 | 9 |
| PT 14b | 13 | 1.6 | 3 | 6 |
| PT 6a | 10 | 1.3 | 3 | 6 |
| PT 5a | 5 | 0.6 | 2 | 3 |
| PT 12 | 7 | 0.9 | 2 | 2 |
| PT 19 | 7 | 0.9 | 2 | 2 |
| PT 25 | 7 | 0.9 | 2 | 2 |
| PT 7a | 7 | 0.9 | 2 | 2 |
| PT 13a | 2 | 0.3 | 1 | 2 |
| PT 29 | 2 | 0.3 | 2 | 2 |
| PT 30 | 2 | 0.3 | 1 | 2 |
| PT 21c | 2 | 0.3 | 1 | 1 |
| PT 13 | 1 | 0.1 | 1 | 1 |
| PT 2 | 1 | 0.1 | 1 | 1 |
| PT 21b | 1 | 0.1 | 1 | 1 |
| PT 24 | 1 | 0.1 | 1 | 1 |
| PT 28 | 1 | 0.1 | 1 | 1 |
| PT 4a | 1 | 0.1 | 1 | 1 |
| PT 4b | 1 | 0.1 | 1 | 1 |
| PT 5c | 1 | 0.1 | 1 | 1 |
| RDNC | 13 | 1.6 | 2 | 8 |
| non typeable | 60 | 7.6 | 6 | 31 |

*S.* Enteritidis phage type four (PT4) was by far the most common reported phage type in the EU and it was isolated in 5 MSs. The second and third most frequently isolated *S.* Enteritidis phage types at EU-level were PT8 and PT7, respectively. They were both also isolated in 5 MSs.

Figure 14 displays for every MS that provided phage type information the most frequently identified holdings in the EU with *S.* Enteritidis phage types.

**Figure 14. Most frequently identified holdings with *Salmonella* Enteritidis phage types in the EU layer survey, 2004 – 2005**




### 4.4.1.2 *S.* Typhimurium phage types

Data on *S.* Typhimurium phage types were provided by seven MSs (Austria, the Czech Republic, Finland, Germany, Italy, the Netherlands and the United Kingdom), whereas eight MSs with *S.* Typhimurium isolates did not report phage typing information.

The MSs that reported information regarding *S.* Typhimurium phage types had 113 *S.* Typhimurium isolates altogether, out of which 73 (64.6%) were phage typed (Table 11). This represented 34.8% of the total 325 *S.* Typhimurium isolates at EU level. In table 11, that presents the most frequently reported phage types, the ranking is based on the percentages of specific *S.* Typhimurium phage type-positive holdings in the EU. MS-specific overviews *S.* Typhimurium phage types are in Annex XI.

**Table 11. Distribution of *S.* Typhimurium phage types in laying hens in the EU, 2004-2005**

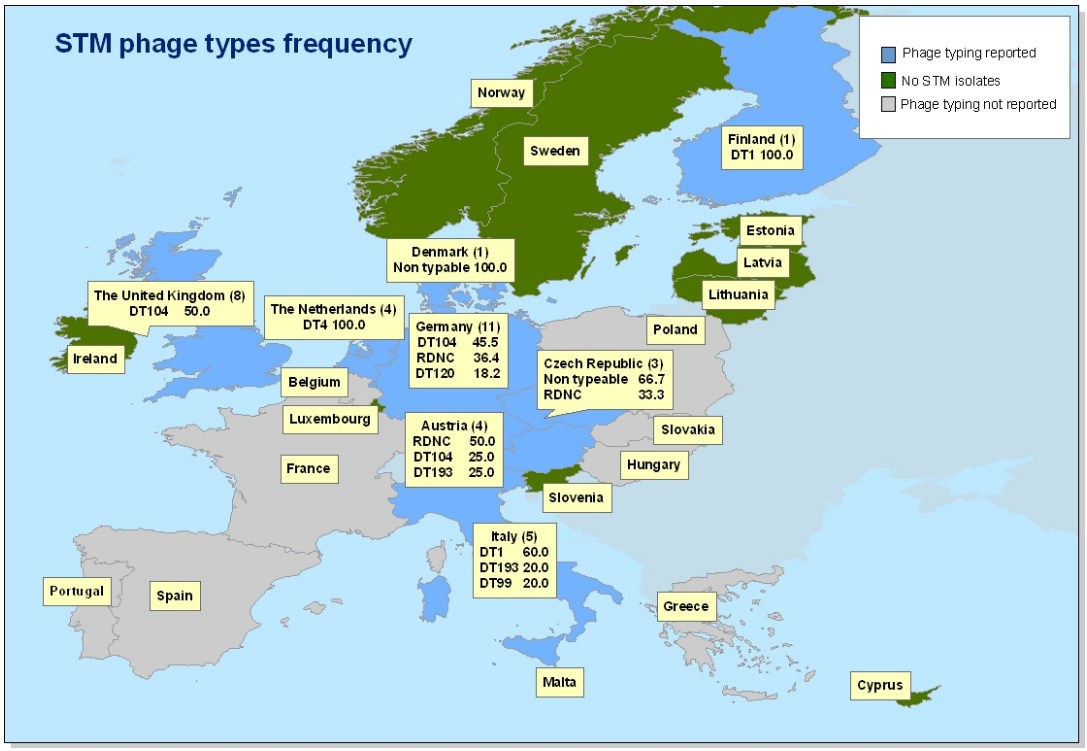
| *S.* Typhimurium (N=73) | | | No of Member States | No of holdings where |
|---|---|---|---|---|
| phage type | N | % | reporting the phage type | the phage type was detected |
| DT 104 | 37 | 50.7 | 3 | 14 |
| DT 1 | 5 | 6.8 | 3 | 5 |
| DT 193 | 4 | 5.5 | 2 | 2 |
| DT 120 | 3 | 4.1 | 1 | 2 |
| DT 49 | 6 | 8.2 | 1 | 1 |
| DT 195 | 1 | 1.4 | 1 | 1 |
| DT 2a | 1 | 1.4 | 1 | 1 |
| DT 56 | 1 | 1.4 | 1 | 1 |
| DT 7 | 1 | 1.4 | 1 | 1 |
| DT 9 | 1 | 1.4 | 1 | 1 |
| DT 99 | 1 | 1.4 | 1 | 1 |
| RDNC | 9 | 12.3 | 3 | 7 |
| non typeable | 3 | 4.1 | 2 | 3 |

RDNC = 'reacts but does not conform' (to a recognised phage lysis pattern)

*S.* Typhimurium definite type 104 (DT104) was the most frequently isolated phage type in the laying hen flocks in the EU, and it was isolated in 3 MSs. The second most frequently isolated *S.* Typhimurium phage type at EU-level was DT1, also isolated in 3 MSs.

Figure 15 displays for every MS that provided phage type information the most frequently identified holdings in the EU with *S.* Typhimurium phage types.

**Figure 15. Most frequently identified holdings with *Salmonella* Typhimurium phage types in the EU layer survey, 2004 – 2005**




### 4.4.2. Testing of antimicrobial susceptibility

Member States could also submit additional information on the antimicrobial susceptibility of *Salmonella* isolates. The study protocol recommended using one isolate per serovar per flock for antimicrobial susceptibility testing, but this varied amongst MS. Quantitative methods and the standards for antimicrobial susceptibility testing given by the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards) for specific antimicrobials were recommended.

Member States reported the tested isolates as susceptible, intermediate, or resistant to the tested antimicrobials. The breakpoints used by the MSs were not harmonised by the study protocol.
Data on the occurrence of antimicrobial resistance in *S.* Enteritidis, *S.* Typhimurium, and/or in serovars other than *S.* Enteritidis and *S.* Typhimurium were provided by 13 MSs (Tables 12-14). Six MSs with *Salmonella* isolates did not report any antimicrobial susceptibility testing results.

The 13 reporting MSs provided data relating to 30 antimicrobials. The results are presented for ten antimicrobials which are regarded as the most important from a public health point of view, or which may be indicative of the clonal spread of resistant *Salmonella* serovars or phage types of *S.* Typhimurium.

The results are presented for *S.* Enteritidis and *S.* Typhimurium isolates, as well as for isolates of serovars other than S. Enteritidis and *S.* Typhimurium combined. All MSs reporting any isolate tested for susceptibility against any of these ten antimicrobials were included in this analysis.

The susceptibility data were collapsed at the holding-level as this is the epidemiological unit of interest. Consequently, the antimicrobial resistance data is reported as the proportion of *Salmonella*-positive holdings having at least one resistant *Salmonella* isolate.

The reporting MSs, with few exceptions, followed the antimicrobial susceptibility testing protocol and tested at least one isolate per serovar per holding. Consequently, isolates were tested from almost all *Salmonella*-positive holdings of the reporting MSs. The reasons why isolates from some positive *Salmonella* holdings were not tested were not provided.

In general, a higher proportion of antimicrobial resistant isolates was reported for *S.* Typhimurium than for *S.* Enteritidis and other serovars.

When data based on less than ten tested holdings is excluded, the highest proportion of holdings with isolates of *S.* Enteritidis resistant to ampicillin (14.3%) and tetracyclines (14.3%) was reported by the United Kingdom, whereas resistance to nalidixic acid was the highest in Poland (10.7%) (Table 12). In a similar way for *S.* Typhimurium, the highest proportion of holdings with resistance amongst isolates was reported by Germany for ampicillin, sulphonamide and tetracycline (in all 63.6%). Italy reported 10% of the isolates to be resistant to nalidixic acid (Table 13). No *S.* Enteritidis or *S.* Typhimurium isolates resistant to enrofloxacin (a fluoroquinolone substance) were reported.

The proportion of holdings with at least one resistant isolate of a serovar other than *S.* Enteritidis and *S.* Typhimurium (Table 14) was the highest in Italy for ampicillin (27.0%), nalidixic acid (39.5%), streptomycin (40.5%) and tetracycline (38.5%). Italy was also the only MS in which resistance to cefotaxime was identified, although most MS did not test for this. No ciprofloxacin resistant isolates were identified, but resistance to gentamicin was found in Denmark. When data based on less than ten holdings was excluded, IT and SI reported a resistance to enrofloxacin of serovars other than *S.* Enteritidis and *S.* Typhimurium of 2.6% and 10% respectively.

**Table 12. Ocurrence of antimicrobial resistance in *Salmonella* Enteritidis isolated from laying hen holdings in the EU, 2004-2005**

| Country | Positive holdings | Tested holdings | | Antimicrobial | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Ampicillin | Cefotaxime | Chloramphenicol | Ciprofloxacin | Gentamicin | Nalidixic acid | Streptomycin | Sulfonamide | Tetracycline | Trimethoprim |
| | N | N test | % | %R | %R | %R | %R | %R | %R | %R | %R | %R | %R |
| Austria | 32 | 32 | 100.0 | 0.0 | | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Belgium[1] | 39 | 31 | 79.5 | 3.2 | | 0.0 | | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Czech Republic[2] | 38 | 38 | 100.0 | 2.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Denmark | 2 | 1 | 50.0 | 0.0 | | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Estonia | 1 | 1 | 100.0 | | | | | 0.0 | 100.0 | 0.0 | | 0.0 | |
| Germany | 126 | 126 | 100.0 | 0.0 | | 0.0 | 0.0 | 0.0 | 3.2 | 0.0 | 0.0 | 0.0 | 0.0 |
| Italy | 15 | 6 | 40.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |
| Lithuania | 4 | 4 | 100.0 | 0.0 | | 0.0 | 0.0 | 0.0 | 25.0 | 0.0 | | 50.0 | 0.0 |
| Poland[3] | 179 | 177 | 98.9 | 0.6 | | 0.0 | 0.0 | 0.0 | 10.7 | 1.1 | 1.7 | 0.0 | 0.6 |
| Slovenia | 9 | 8 | 88.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| The Netherlands | 25 | 24 | 96.0 | | 0.0 | 0.0 | 0.0 | 0.0 | 4.2 | | 0.0 | 0.0 | 0.0 |
| The United Kingdom | 28 | 28 | 100.0 | 14.3 | 0.0 | 0.0 | 0.0 | 0.0 | 3.6 | 3.6 | 7.1 | 14.3 | |

1. For Belgium; N test=30 for Sulfonamide and N test=1 for Trimethoprim

2. For Czech Republic; N test=37 for Sulfonamide

3. For Poland; N test=94 for Ciprofloxacin and N test=175 for Trimethoprim

**Table 13. Occurrence of antimicrobial resistance in *Salmonella* Typhimurium isolated from laying hen holdings in the EU, 2004-2005**

| COUNTRY | Positive holdings | Tested holdings | | Antimicrobial | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Ampicillin | Cefotaxime | Chloramphenicol | Ciprofloxacin | Gentamicin | Nalidixic acid | Streptomycin | Sulfonamide | Tetracycline | Trimethoprim |
| | N | N test | % | %R | %R | %R | %R | %R | %R | %R | %R | %R | %R |
| Austria | 4 | 4 | 100.0 | 0.0 | | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Belgium | 1 | 1 | 100.0 | 0.0 | | 0.0 | | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |
| Czech Republic | 3 | 3 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Denmark | 1 | 1 | 100.0 | 0.0 | | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Finland | 1 | 1 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | | 0.0 | 0.0 | | 0.0 | |
| Germany | 11 | 11 | 100.0 | 63.6 | | 36.4 | 0.0 | 0.0 | 0.0 | 54.5 | 63.6 | 63.6 | 0.0 |
| Italy[1] | 14 | 10 | 71.4 | 20.0 | 0.0 | 10.0 | 0.0 | 0.0 | 10.0 | 20.0 | 22.2 | 20.0 | |
| Poland[2] | 7 | 7 | 100.0 | 28.6 | | 14.3 | 0.0 | 0.0 | 57.1 | 42.9 | 42.9 | 28.6 | 0.0 |
| The Netherlands | 7 | 7 | 100.0 | | 0.0 | 28.6 | 0.0 | 0.0 | 0.0 | | 71.4 | 57.1 | 28.6 |
| The United Kingdom | 8 | 8 | 100.0 | 50.0 | | 50.0 | 0.0 | 0.0 | 12.5 | 50.0 | 50.0 | 50.0 | |

1. For Italy; N test=9 for Sulfonamide

2. For Poland; N test=3 for Ciprofloxacin

**Table 14. Occurrence of antimicrobial resistance in serovars other than *Salmonella* Enteritidis and *Salmonella* Typhimurium isolated from laying hen holdings in the EU, 2004-2005**

| Country | Positive holdings | Tested holdings | | Antimicrobial | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Ampicillin | Cefotaxime | Chloramphenicol | Ciprofloxacin | Gentamicin | Nalidixic acid | Streptomycin | Sulfonamide | Tetracycline | Trimethoprim |
| | N | N test | % | %R | %R | %R | %R | %R | %R | %R | %R | %R | %R |
| Austria | 18 | 18 | 100.0 | 5.6 | | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Belgium | 28 | 23 | 82.1 | 8.7 | | 0.0 | | 0.0 | 0.0 | 4.3 | 8.7 | 4.3 | |
| Czech Republic | 5 | 5 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 20.0 | 20.0 | 20.0 | 0.0 |
| Denmark[1] | 2 | 2 | 100.0 | 0.0 | | 0.0 | 0.0 | 50.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Estonia | 1 | 1 | 100.0 | 0.0 | | | 0.0 | 0.0 | | | | 100.0 | |
| Germany | 82 | 82 | 100.0 | 0.0 | | 0.0 | 0.0 | 0.0 | 2.4 | 4.9 | 1.2 | 2.4 | 0.0 |
| Italy[2] | 87 | 42 | 48.3 | 27.0 | 2.6 | 0.0 | 0.0 | 2.4 | 39.5 | 40.5 | 13.5 | 38.5 | |
| Latvia | 1 | 1 | 100.0 | | | 0.0 | 0.0 | 0.0 | 0.0 | | | | |
| Lithuania | 1 | 1 | 100.0 | 0.0 | | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | 100.0 | 100.0 |
| Poland[3] | 113 | 111 | 98.2 | 6.3 | | 0.0 | 0.0 | 0.0 | 18.0 | 9.0 | 7.2 | 4.5 | 0.0 |
| Slovenia | 10 | 10 | 100.0 | 10.0 | 0.0 | 0.0 | 0.0 | 0.0 | 10.0 | 10.0 | 10.0 | 10.0 | 0.0 |
| The Netherlands | 35 | 32 | 91.4 | | 0.0 | 0.0 | 0.0 | 0.0 | 12.5 | | 3.1 | 3.1 | 3.1 |
| The United Kingdom | 21 | 18 | 85.7 | 0.0 | | 0.0 | 0.0 | 0.0 | 0.0 | 5.6 | 5.6 | 0.0 | |

1. For Denmark; N test=1 for Sulfonamide

2. For Italy; N test=37 for Ampicillin, N test=38 for Cefotaxime, N test=35 for Cloramphenicol, N test=35 for Ciprofloxacin N test=38 for Nalidixic acid, N test=37 for Streptomycin, N test=37 for Sulfonamide and N test=39 for Tetracycline

3. For Poland; N test=59 for Ciprofloxacin

# 5. Discussion

## 5.1. Observed prevalence of *Salmonella*

### 5.1.1. Observed prevalence in the Member States

The EU-weighted observed prevalence of *Salmonella*-positive holdings, calculated on the basis of the final dataset, was substantially similar to the prevalence reported in the Preliminary Report. This was also the case for most individual MSs. Only a small number of MSs experienced an important change in their prevalence figures as a result of more holdings being included in the final dataset. The increased number of holdings in the final cleaned dataset provided improved data quality, and facilitated a more precise estimation of the observed prevalence of *Salmonella*-positive holdings and narrower confidence intervals.

Despite this, differences in the MS-specific prevalences were considerable, with the result that an EU weighted mean can be regarded as arbitrary and of little practical use. For example, the observed *Salmonella* spp. and observed *S.* Enteritidis/*S.* Typhimurium holding prevalences at the MS level range from 0% to 79.5% and 0% to 62.5%, respectively.

In general, the observed prevalences of both *Salmonella* spp. and *S.* Enteritidis/*S.* Typhimurium in the MSs in this study, were substantially higher than those reported by the MSs for laying hens in the national zoonoses reports in previous years, as well as for the regular monitoring results from 2005 (Community Summary Report on Zoonoses 2005[1]). This might be the result of a more sensitive sampling design applied in the baseline study. Indeed, the number of samples taken from a poultry house was generally higher, and the volume and variety of sample material collected was greater, than was normally the case for most MSs. Furthermore, the baseline study specifically investigated flocks at the end of their production period, when the within-flock *Salmonella* prevalence is often at its highest, whereas the prevalence in laying hens reported in the Community zoonoses report covers all age groups (day-old chicks, rearing, and all stages of production). Another additional reason for the observed difference between the regular monitoring results and the outcome of the baseline study may be the target population addressed. In the baseline study, only holdings with at least 1,000 birds were targeted, whereas there are many holdings in many Member States that have fewer birds. If these latter holdings are included in the regular monitoring, this could also explain some of the differences observed. However, it is noteworthy that infected flocks were unexpectedly identified in some MSs with very active surveillance and control programmes. Additional factors may be the increased sensitivity of the ISO 6579 Annex D test method used, and the fact that the competent authorities and NRLs were responsible for sample collection and testing.

[1] The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2005, *The EFSA Journal* (2006), 94.

### 5.1.2. Consistency with respect to the sampling design

The study design, where there was sampling of only one flock per holding, proved not to be optimal for estimation of prevalence for two main reasons:

- An important bias was introduced by the fact that the sub-population of flocks from holdings with more than one flock is under-represented. As a consequence, the observed prevalence in this study must be considered a minimum, and is likely to be significantly higher in reality.
- The flock prevalence cannot be estimated without any further assumptions or additional information as only one flock per holding was sampled.

As an alternative, a multi-level sampling design (including sampling more than one flock per holding) could be considered for upcoming baseline studies to minimize this bias.

The underestimation of the observed holding prevalence can be explained by the fact that, since only one flock per holding was sampled, flocks on smaller holdings are more likely to be sampled compared to flocks on larger holdings. But, assuming larger holdings are more likely to be infected as indicated by the results, this would result in estimates of the overall flock prevalence that are underestimated if the estimator is not adjusted. Since there are few published EU MS reports of the clustering of *Salmonella* on commercial laying farms, the uncertainty surrounding the results of any simulation is substantial.

It should be kept in mind that a flock found positive in this study does not necessarily mean a flock including currently infected birds because the samples were collected from the environment of the birds (faecal droppings and dust). However, these types of environmental samples have proved to be sensitive indicators of *Salmonella* infection in a flock, even though the current within-flock prevalence may be low[1]. In such cases, the relevance of positive environmental samples in terms of the risk of contamination of eggs at the point of sale is also unclear, and more research is needed to elucidate this.

Substantial numbers of holdings were positive for *S.* Enteritidis/*S.* Typhimurium in only one or two samples from the seven samples taken. This may indicate that a reduction in the number of samples taken from a flock might reduce the numbers of flocks found positive. However, direct comparison between different numbers of environmental samples is difficult, because fewer samples would normally be collected from a larger area of the poultry house, whereas the individual survey samples were taken from separate sectors of the house.

In addition, as indicated by simulation, the number of samples taken and the type of sample has an obvious influence on the sensitivity of the sampling scheme, and thereby the accuracy of the prevalence estimates. This may be reflected in the accuracy of prevalence estimates from future routine monitoring of laying flocks. Decreasing the number of faeces and/or dust samples per holding could lead to a lower prevalence estimate. The decrease of dust samples had the largest impact. However, simulation results in this study show that as long as at least one dust sample is included, there is only a marginal change in prevalence when decreasing the sample size from seven to four samples per flock.

[1] EFSA (2004) Opinion of the Scientific Panel on Biological Hazards on the use of vaccines for the control of *Salmonella* in poultry. *The EFSA Journal* (2004) 114, 1-74.

## 5.2. Frequency of isolated *Salmonella* serovars

*S.* Enteritidis was clearly the predominant serovar in laying hen flocks in the EU, even though the frequency of its isolation varied considerably amongst the MSs. Strong variation was also the case for the second and third most frequently isolated serovars (*S*. Infantis and *S.* Typhimurium).

The predominance of *S*. Enteritidis was expected because of its unique ability to colonise, for long-term, the ovary and the oviduct of laying hens, and its capacity to persist between consecutively housed flocks in the environment and in the rodent population. The relative frequency of *S*. Infantis and *S.* Typhimurium can be explained by a similar, but reduced, capacity for vertical transmission and the historic occurrence of these serovars in layer breeding flocks in some MSs.

*S*. Enteritidis, *S*. Typhimurium and *S*. Infantis were also the 3 most frequently reported serovars from human salmonellosis cases in the EU during 2005 with the isolation shares of 52.2%, 9.1% and 0.8% respectively (Community Summary Report on Zoonoses 2005). Seven out of the ten most frequently reported *Salmonella* serovars from human cases are amongst the 17 most frequently isolated serovars from laying hens. Thus, the serovar distributions found in human salmonellosis cases and in laying hen populations have many similarities. However, in the case of serovars such as *S*. Typhimurium and *S*. Infantis, other farm animals species such as pigs, turkeys and cattle may be more important reservoirs. Other poultry, such as broilers, and foreign travel, may also be implicated in human *S*. Enteritidis infection.

## 5.3. Analysis of factors associated with *Salmonella* positivity

To ensure proper understanding of statistical analyses performed, it should be noted that:

- the potential factors evaluated are those associated with an increased probability of a positive sample (i.e. *Salmonella* positivity), meaning that a significant statistical correlation can be observed. But these associations do not necessarily indicate a causal relationship between the factor and the observed outcome,
- the potential risk factors that were evaluated were not comprehensive, and no interaction effects were investigated,
- spatial and temporal factors were not accounted for in the analysis.

These limitations are due to the study design and the nature of the data received. As a consequence, the regression analysis can only generate hypotheses for potential risk factors which may have been associated with the presence of *Salmonella* in the samples.

### 5.3.1. Variability between Member States

An important finding of the regression analysis was that the associated factors, as well as the strength of the associations, differ when data is analysed at the EU level and at the MS group level. Both analyses may be important for EU risk managers, while the latter seems more appropriate from an epidemiological point of view and is also relevant to the development of national control plans. Differences in risk factors were also observed between the MS groups with different levels of prevalence.

### 5.3.2. Discussion of the analysis results valid for all *Salmonella* serovars

Dust samples were consistently more likely to be positive overall, compared to faeces samples. This applied to all the *Salmonella* serovars analysed and at both EU level and for all the MS groups. The higher chance of identifying *Salmonella* in dust samples may be explained by the survival of *Salmonella* serovars in dust. *Salmonella* is relatively more resistant to desiccation than many competitor organisms[1] [2] [3], so isolation from dust is often easier than from fresh faeces. Dust is also a better indicator of previous peaks of *Salmonella* excretion or intermittent excretion in the flock. This may be a factor explaining the higher recovery of serovars other than *S*. Enteritidis and *S*. Typhimurium from dust as these serovars are commonly found from feed (Community Summary Report on Zoonoses 2005) and thus may give rise to short-lived infection in flocks. Contaminated feed particles may also contribute to the dust samples.

The flock production type was always associated with the occurrence of *Salmonella* in analyses of all serovar–MS combinations. Cage flocks were normally associated with the highest *Salmonella* prevalence. However, *Salmonella* prevalences in barn and organic types of flock did not always differ significantly from cage flocks in the two MS group analyses, indicating that different risk factors are likely to apply in high prevalence situations.

Cage flocks typically belonged to the larger holding and flock size categories, and there was a strong correlation between the flock type and the size characteristics. Thus, holding size might be a major risk factor for *Salmonella* infection. In smaller holdings the susceptible animal population is smaller throughout the year, which might contribute to a reduced risk of introduction or maintenance of *Salmonella* infection. Smaller holdings are also likely to have a single or limited number of houses operated on an all-in/all-out basis. However, the results do not necessarily indicate that it are the size variables that are the actual risk factors, but some underlying mechanism – maybe related to the production type. In general, the higher prevalence in cage flocks might partly be explained by the fact that hens in the more intensive systems have a higher risk of being infected due to a relatively large flock size and higher density of hens. Moreover, cages can be difficult to disinfect and the housing may harbour breeding populations of rodents and other potential vectors such as flies or litter beetles[4]. *Salmonella* has been shown to be more persistent in consecutive cage flocks compared with non-cage flocks in which the infection is more easily cleaned out during the empty period between flocks[5].

No significant association was found between the occurrence of *Salmonella* and the flock age type (homogeneous or mixed age flocks), nor with the medication status (antimicrobials used within the last two weeks). Mixed age flocks are more likely to be held on smaller holdings and the antimicrobials normally used during lay (for example, tetracyclines, tylosin or tiamutalin) have minimal effect on the excretion of *Salmonella*.

[1] Davies, R.H. and Wray, C. (1996) Persistence of *Salmonella enteritidis* in poultry units and poultry feed. *British Poultry Science* 37, 589-596.
[2] Davies, R.H. and Breslin, M. (2003) Persistence of *Salmonella* Enteritidis PT4 in the environment and arthropod vectors on an empty free-range chicken farm. *Environmental Microbiology* 5, 79-84.
[3] Miura, S., Sato, G., and Miyamae, T. (1964) Occurrence and Survival of *Salmonella* Organisms in Hatcher Chick Fluff from Commercial Hatcheries. *Avian Diseases*, Vol. 8, No. 4, pp. 546-554.
[4] Davies, R.H. and Wray, C. (1994) *Salmonella* pollution in poultry units and associated premises. In *Pollution in livestock production systems.* (ed. I. Ap Dewi, R.F.E. Axford, I. Fayez, M. Marai, and H. Omed), pp. 137-165. CAB International.
[5] Davies, R.H. and Breslin, M. (2003) Observations of *Salmonella* contamination of commercial laying farms before and after cleaning and disinfection. *Veterinary Record* 152, 283-287.

### 5.3.3. Specific results for *Salmonella* Enteritidis

At the global EU level, vaccination of flocks with either *S.* Enteritidis or non-*S.* Enteritidis type of vaccines was found to decrease the risk of *S.* Enteritidis positivity when compared to unvaccinated flocks. This was also the case for the MSs group having the highest prevalence, whereas vaccination was not associated with *S.* Enteritidis positivity in the intermediate prevalence MS group. This may indicate that vaccination is a beneficial control measure for laying-hen populations with a moderate to high *Salmonella* prevalence. This is consistent with the opinion of the Biological Hazard Panel on the use of vaccines for the control of *Salmonella* in poultry[1]. The opinion states that if a control programme is targeting for serovars *S.* Enteritidis and *S.* Typhimurium in laying hens and the flock prevalence is high, vaccination may be useful in reducing shedding and egg contamination. If the flock prevalence is low, vaccination may not be so useful but could still be used as one of the preventive measures to maintain a low prevalence. Generally other preventive and control measures beside vaccinations are needed in successful control of *Salmonella* infections in laying hen flocks.
It is noteworthy that there were two countries in the study where vaccination is forbidden and where no *Salmonella* was isolated (Sweden and Norway).
Lastly, the results of the baseline study indicated that vaccinations against serovars other than *S.* Enteritidis also seem to protect flocks against *S.* Enteritidis.
A second significant risk factor associated with the occurrence of *S.* Enteritidis was the age of the hens present in the flock at the time of sampling. This is likely to reflect an increase in the susceptibility of the hens as they reach the end of their productive life, as well as a waning of vaccinal protection and increasing populations of red-mite and *Salmonella* vectors such as rodents.

### 5.3.4. Specific results for *Salmonella* Typhimurium and serovars other than *Salmonella* Enteritidis and *Salmonella* Typhimurium

No significant factors were associated with the occurrence of *S.* Typhimurium other than the sample type and flock production type. This is different from the results for *S.* Enteritidis and the other serovars. Although this might partly be explained by the lower observed prevalence of *S.* Typhimurium, it does indicate the possibility of a differing epidemiology in the *Salmonella* serovars in laying hen flocks. *S.* Typhimurium is found in a variety of potential sources such as other farm animal species, free-living wild birds, and feed. There is also less regular use of specific *S.* Typhimurium vaccination in most MSs. These may result in more persistent infections of flocks.

In the case of flocks where serovars other than *S.* Enteritidis and *S.* Typhimurium were found, a number of additional associated factors were observed. These included the age of the hens present in the flock at the time of sampling. This factor was similar to that found with *S.* Enteritidis. In addition, vaccination against any *Salmonella* serovar seemed to partially protect flocks against serovars other than *S.* Enteritidis and *S.* Typhimurium. But this observation might also be explained by the fact that farmers who are vaccinating flocks against *Salmonella* may have introduced aslo other control measures against *Salmonella* which would help to protect the flock from infection.

[1] EFSA (2004) Opinion of the Scientific Panel on Biological Hazards on the use of vaccines for the control of *Salmonella* in poultry. *The EFSA Journal* (2004) 114, 1-74.

There were also indications of a possible seasonal effect for serovars other than *S*. Enteritidis and *S*. Typhimurium because there were significant differences between the prevalences in different months. Interestingly, this seasonal effect was only observed for these other serovars. Another factor associated only with the occurrence of the other serovars was an increased delay between the sampling date and the start of bacteriological testing, although a reduction was only apparent when there was a delay of four days or more.

The significant association of the apparent seasonal effect and the effect of an increased delay before testing only with serovars other than *S.* Enteritidis and *S.* Typhimurium is a new finding. The seasonality findings may indicate that *S.* Enteritidis and *S.* Typhimurium infect the flocks more persistently and less seasonally than do the other serovars. It may also be that *S.* Enteritidis and *S.* Typhimurium are excreted in higher numbers so dust samples may remain positive for a longer time. The apparent seasonality in the occurrence of the other serovars might be associated with an increased risk of feed contamination (feed being a likely source of these 'other' serovars[1]) in the autumn months when there is more risk of condensation in feed mills and when the new season's grain, which is more likely to be contaminated, is being used[2].

## 5.4. Analysis of additional information reported on a voluntary basis

### 5.4.1. *Salmonella* Enteritidis and *Salmonella* Typhimurium phage types

Eight out of 18 MSs with *S.* Enteritidis isolates and seven out of 15 MSs with *S.* Typhimurium isolates provided information on phage typing of the isolates. Overall, only approximately one-third of the isolates in the study were phage typed. It was, therefore, not possible to carry out a representative analysis of the *S.* Enteritidis or *S.* Typhimurium phage types from laying flocks in the EU.

*S*. Enteritidis phage type (PT) 4 was the most frequently reported phage type from human salmonellosis cases (Community Summary Report on Zoonoses 2005). This phage type (PT) 4 was also the most frequently reported in this study. In addition, nine of the ten most frequently reported *S*. Enteritidis phage types in humans are amongst the 15 most frequently isolated phage types in the baseline study.

When comparing the *S*. Typhimurium phage types in a similar manner, the same phage type, definitive type (DT) 104, is the most frequently reported in both human cases and laying hen flocks, although this type is commonly reported in other farm animal species. Only four of the ten most frequently reported *S*. Typhimurium phage types in humans are amongst the 12 most frequently isolated phage types from the laying hen study.

Thus *S*. Enteritidis populations isolated from laying hens seem to have more similarities with the isolates from human cases than *S.* Typhimurium populations. This may reflect the fact that *S*. Typhimurium is found in other animal species.

[1] Community Summary Report on Zoonoses 2005

[2] Davies, R.H. and Hinton, M.H. (2000) *Salmonella* in animal feed. In: *Salmonella in domestic animals.* (Eds.) C. Wray, A.Wray. CAB International, Oxford, England, 285-300

### 5.4.2. Antimicrobial susceptibility testing

Thirteen MSs, which is about two-thirds of the MSs which isolated *Salmonella*, reported on antimicrobial susceptibility testing. In general, a higher proportion of antimicrobial resistant isolates was reported for *S.* Typhimurium than for *S.* Enteritidis and other serovars. However, since certain MSs that did not report had a high *Salmonella* prevalence, no representative analysis of the antimicrobial susceptibility of *Salmonella* isolates from laying flocks at the EU level could be made. Moreover, when comparing the results of antimicrobial susceptibility testing of *Salmonella* isolates, attention should be given to the variation in breakpoints used by the MSs, as this renders any comparison between MSs even more difficult.

The proportion of *Salmonella*-positive laying hen holdings with resistant isolates appears to be low compared with the antimicrobial resistance data reported by the MSs for *Gallus gallus* in the national zoonoses reports for previous years, as well as for the year 2005 (Community Summary Report on Zoonoses 2005). This could be explained by the difference in animal populations and in the use of antimicrobials in those populations. The isolates collected in the framework of the baseline study are environmental samples from laying hen flocks with hens at the end of their production period, whereas the *Gallus gallus* population reported on by MSs in the Community Summary report originate from a much more varied population of birds of all age and production groups (day-old chicks, breeding, rearing and production of both laying and meat birds). Isolates from clinical cases are also reported from some MSs. The use of antimicrobials in laying hens in most MSs is very limited because of the age of the birds and the requirement to withhold eggs from sale during and after treatment. This limited usage, together with the occurrence of *Salmonella* serovars which are less likely than average to develop resistance, could partially explain the lower resistance in isolates from reporting MSs.

## 5.5. Relevance of the findings to human salmonellosis

*Salmonella* is an important cause of foodborne disease in humans throughout the world and is a significant cause of morbidity, mortality and economic loss.[1,2,3,4,5] Illness can range from a mild to severe gastroenteritis and in some people, invasive disease, which can be fatal. Long term sequelae such as reactive arthritis can also result from *Salmonella* infections.

In 2005, the reported number of cases and incidence of human salmonellosis in the EU were, respectively, 176,395 cases and 38.2 cases per 100,000 inhabitants (Community Summary Report on Zoonoses 2005). Eggs are considered the predominant source of human salmonellosis in

[1] Roberts JA, Sockett PN. (1994) The socio-economic impact of human *Salmonella* enteritidis infection. International Journal of Food Microbiology 21:117-129.

[2] Adak GK, Long SM, O'Brien SJ. (2002) Trends in indigenous foodborne disease and deaths, England and Wales: 1992-2000. Gut 51: 832-841.

3 Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV. (1999) Food related illness and death in the United States. Emerging Infectious Disease 5: 607-625.

4 Schroeder CM, Naugle AL, Schlosser WD, Hogue AT, Angulo FJ, Rose JS, Ebel ED, Disney WT, Holt KG, Goldman DP. (2005) Estimate of illness from *Salmonella* enteritidis in eggs, United States, 2000. Emerging Infectious Diseases 11: 113-115.

5 Voetsch AC, Van Gilder TJ, Angulo FJ, Farley MM, Shallow S, Marcus R., Cieslak PR, Deneen VC, Tauxe RV. (2004) FoodNet estimate of the burden of illness caused by non-typhoidal *Salmonella* infections in the United States. Clinical Infectious Disease 38: S127-134.

Europe as well as many other countries worldwide[1]. In the EU, eggs and egg products were the most frequently reported source of foodborne outbreaks caused by *Salmonella* in 2005 (Community Summary Report on Zoonoses 2005).

Of the more than 2,500 serovars of *Salmonella enterica*, *S.* Enteritidis is the serovar most often associated with eggborne infections in human, and is also the serovar causing more than 50% of the human *Salmonella* infections in the EU. The second most reported serovar in humans is *S.* Typhimurium, which is less often associated with the consumption of hens' eggs (Community Summary Report on Zoonoses 2005).

The emergence of *S.* Enteritidis over the past 20-25 years in both table-egg laying hens and humans has been explained by the combination of two main factors: the extraordinary epidemiology of *S.* Enteritidis infections in laying hens and the centralised rearing of breeding stock.[2] In contrast to most other zoonotic *Salmonella* serovars, *S.* Enteritidis has been shown to be able to cause a lifelong colonisation of the peri-reproductive tissue of the laying hens. This may lead to colonisation of the egg content during the formation of the egg in the reproductive tract. Due to this ability of vertical transmission, parent stock can transmit the infection to their progeny and laying hens can infect the content of eggs produced for consumption. Amongst the isolated *Salmonella* spp. in this study, also *S.* Typhimurium can be transmitted vertically while vertical transmission of the other *Salmonella* serovars is more unusual[3].

The likelihood of eggs produced by a *Salmonella* infected flock depends, amongst other factors, on flock prevalence, within-flock prevalence, the numbers of organisms harboured and excreted by birds, the frequency with which infected hens lay contaminated egg, and the hygienic conditions in the laying house and egg handling facilities. In naturally *Salmonella* infected laying hens flocks the proportion of infected eggs that are laid varies[4], but many studies show this proportion to be low (often below 3%) [5, 6, 7, 8, 9, 10].

However, considering the large amount of eggs produced for human consumption and the fact that eggs are often pooled in dishes that are consumed lightly cooked, the presence of *Salmonella* in eggs even at a very low level poses a significant risk for human health. Thorough cooking of the eggs will destroy the *Salmonella* and thus eliminates the risk from the food itself. Besides the consumption of raw or undercooked eggs or egg products, also cross contamination during food

1 SCVPH, (2003). Opinion of the Scientific Committee on Veterinary Measures relating to Public Health on *Salmonellae* in Foodstuffs. Adopted on 14-15 April.

2 Thorns, C. J. (2000) Bacterial food-borne zoonoses. Rev.sci.tech.Off.int.Epiz. 19:226-239.

3 EFSA (2005) Opinion of the Scientific Panel on Biological Hazards on the request from the Commission related to the Microbiological risks on washing of table eggs. *The EFSA Journal* (2005) 269, 1-39.

4 Humphrey, T.J., Baskerville, A., Mawer, S., Rowe, B., and Hopper, S., (1989) *Salmonella enteritidis* phage type 4 from the contents of intact eggs: A study involving naturally infected hens. Epidemiology and Infection 103: 415-423.

5 Humphrey TJ, Whitehead A, Gawler AH, Henley A, Rowe B. Numbers of *Salmonella enteritidis* in the contents of naturally contaminated hens' eggs. Epidemiol Infect. (1991) Jun;106(3):489-96.

6 de Louvois, J. (1993) *Salmonella* contamination of eggs: a potential source of human salmonellosis. PHLS Microbiology Digest, 10 (3), 158-162.

7 Henzler, D.J., Ebel, E., and Sanders, J. (1994) *Salmonella enteritidis* in Eggs from Commercial Chicken Layer Flocks Implicated in Human Outbreaks. Avian Diseases 38: 37-43.

8 Kinde, H., Read, D.H., and Gardner, I.A. (1996) *Salmonella enteritidis*, phage type 4 infection in a commercial layer flock in southern California: Bacteriologic and epidemiologic findings. Avian Diseases 40: 665-671.

9 Schlosser, W.D., et al. (1999 The *Salmonella enterica* serovar Enteritidis Pilot Project. p.353-365, *In:* A.M. Saeed, R.K. Gast and M.E. Potter (eds). *Salmonella enterica* serovar enteritidis in humans and animals: Epidemiology, pathogenesis, and control. Ames, Iowa IA: Iowa State University Press.

10 Advisory Committee on the Microbiological Safety of Food (2001). Second Report on *Salmonella* in Eggs. The Stationery Office. ISBN 0-11-322466-4.

preparation is a possible route of infection. There is also a risk of cross-contamination of other food products by organisms from eggshells or spilled contents which are handled during the preparation of egg dishes. It is therefore important to reduce the risk of egg *Salmonella* contamination and the numbers of *Salmonella* bacteria present.

In conclusion, the results of this study show that *Salmonella enterica*, particularly *S.* Enteritidis, occurs in the commercial large-scale laying hen production in the EU, but at varying levels in Member States, and strongly indicates that table eggs continue to be an important source of human salmonellosis in EU.

# 6. Conclusions

- The observed holding prevalence of *Salmonella* spp. varied widely amongst MSs from 0% to 79.5%. The weighted prevalence of *Salmonella* spp. in laying hen holdings within the EU was estimated to be 30.8%. The weighted prevalence of *S.* Enteritidis and *S.* Typhimurium was 20.4%.
- The study suffered from a design bias that underestimated the holding observed prevalence. The observed prevalences are likely to be higher in reality, especially in larger holdings. This means that a substantial proportion of eggs in many MSs may originate from holdings where *Salmonella* is present.
- Though the observed *Salmonella* prevalences in this study are likely to be underestimated, they are generally higher than those reported in national zoonoses reports by the MS, when a variety of monitoring methods are used.
- The most frequently reported serovar was *S.* Enteritidis, which represented approximately half of the isolates at the EU level, and was found in 18 MSs. *S.* Enteritidis is also the predominant serovar in human salmonellosis cases. This supports suggestions that contaminated eggs are likely to be the major source of human infection.
- The nature of the study design only allowed for investigation of potential risk factors. The results of the analysis of factors associated with the occurrence of *Salmonella* were also influenced to an extent by the design bias.
- The findings concerning factors associated with the occurrence of *Salmonella* were sometimes different at the EU level compared with those for the MS groups with similar prevalences. Also, there were differences between MS groups having different levels of observed *Salmonella* prevalence.
- Dust samples are significantly more likely to identify *Salmonella*-positive holdings than faeces samples.
- Cage flock holdings are more likely to be contaminated with *Salmonella*. While cage flocks were of larger size this means as well that the larger size holdings and flocks were more likely to be positive.
- There are indications that factors associated with the occurrence of *Salmonella* may depend on the specific *Salmonella* serovar.
    - *S.* Enteritidis and *S.* Typhimurium show no significant variations in prevalence at different times of the year, whereas other serovars show more apparent seasonal variation.
    - Vaccination against *Salmonella* is associated with a lower risk of finding *S.* Enteritidis and serovars other than *S.* Enteritidis and *S.* Typhimurium, suggesting some possible cross-protection. However no significant effect was observed with *S.* Typhimurium.
- The phage typing and antimicrobial susceptibility testing information was non-representative because of incomplete reporting. However, many *S.* Enteritidis and *S.* Typhimurium phage types which occur commonly in human salmonellosis cases were also frequently found in samples from laying hen flocks. This was especially the case for *S.* Enteritidis phage types.
- The antimicrobial resistance amongst the *Salmonella* isolates from laying hen flocks seemed low.

- Although a low proportion of eggs deriving from *Salmonella*-infected laying hen flocks are normally contaminated with *Salmonella* at the point of sale, the presence of *Salmonella* within eggs and on shells presents a significant public health threat. The level of this risk is likely to vary in accordance with the observed *Salmonella* prevalence in the MSs.

# 7. Recommendations

- Control measures to reduce the *Salmonella* shedding in laying hen flocks and the egg contamination would be essential to decrease the number of human salmonellosis cases in the EU. Vaccination against *S.* Enteritidis would be one of the useful control measures available.
- Improved validation during the data submission period as well as immediate feedback to MSs regarding submitted non-valid data values could reduce the need for excessive data exclusion during the validation process.
- More quantitative field studies involving the sampling of multiple flocks per holding are necessary to estimate the clustering of positive flocks on holdings and to determine the true baseline prevalence of *Salmonella.*
- Follow up studies are necessary to:
    - confirm the identified potential risk factors,
    - disentangle the effects of the risk factors - flock production types, size variables (number of hens in a flock and on the holding, number of flocks per holding), age of birds at sampling – all of which may vary systematically between MSs with different prevalences of *Salmonella*,
    - determine the influence of the sampling regimes used in the different types of holding.
- A meta-analysis of risk factor analysis studies carried out in MSs could be carried out to investigate the effects of specific vaccination regimens as this could provide useful evidence for enhanced future control programmes. Other risk factors may also be examined with more analytical power using this approach. It is also necessary to confirm major risk factors by applying controlled intervention studies.
- The additional information reported on a voluntary basis (phage typing and antimicrobial susceptibility testing) should be mandatory if representative information is needed. Testing of smaller representative panels of isolates, rather than isolates from all holdings in MSs with a high prevalence, would be sufficient to investigate differences between MSs and to monitor trends.

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# Abbreviations

| | |
|---|---|
| CI | Confidence Interval |
| EEA | European Economic Area |
| EFSA | European Food Safety Authority |
| EU | European Union |
| MS(s) | Member State(s) |
| SE | *Salmonella* Enteritidis |
| STM | *Salmonella* Typhimurium |
| Non-SE/STM | Non-*Salmonella* Enteritidis/*Salmonella* Typhimurium serovar(s) |

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# Annexes

**Annex I. List of criteria used to identify non-valid and non-plausible information in the *Salmonella* laying hens database**

The variables are uniquely identified using the 'item integer' mentioned in the ad hoc Data Dictionary.

**Step 1**

In step one records (samples) were selected.

| Criterion No | **Rationale for the criterion** |
|---|---|
| 1 | 007 Hens in holding: < 30<br>This criterion selects all records containing less than 30 hens in the holding. |
| 2 | 007 Hens in holding: < values for 011 Number of hens in flock<br>This criterion selects all records containing a number of hens in the holding that is smaller than the number of hens in the flock. A 10% difference (in case '007 hens in holding' is a smaller number compared to '011 Number of hens in flocks') is allowed. |
| 3 | 008 Number of flocks: <= 0<br>This criterion selects all records containing a number of flocks equal to, or lower than, zero. |
| 4 | 008 Number of flocks: > 40<br>This criterion selects all records containing a number of flocks higher than 20. |
| 5 | 008 Number of flocks: values: = 1 and value for 007 Hens in holding IS NOT EQUAL TO value for 011 Number of hens in flock<br>This criterion selects all records with one flock in the holding where the number of hens in that holding does not equal the number of hens in the flock. A 10% difference in either direction is allowed. |

| Criterion No | **Rationale for the criterion** |
|---|---|
| 6 | 010 Date of sampling: < 15 September 2004 |
| | This criterion selects all records containing a date of sampling before 15 September 2004. |
| 7 | 010 Date of sampling: > 15 October 2005 |
| | This criterion selects all records containing a date of sampling after 15 October 2005. |
| 8 | 011 Number of hens in flock: < 30 |
| | This criterion selects all records containing flocks with less than 30 hens. |
| 9 | 013 Age of hens at sampling: > 150 |
| | This criterion selects all records containing hens aged more than 150 weeks. |
| 10 | 013 Age of hens at sampling: < 30 and 014 Maximum age of hens at sampling: IS NULL |
| | This criterion selects all records containing hens aged less than 30 weeks in homogeneous age flocks. |
| 11 | 013 Age of hens at sampling: <= 0 |
| | This criterion selects all records containing hens aged zero weeks or less. |
| 12 | 014 Maximum age of hens at sampling: > 150 |
| | This criterion selects all records containing hens aged more than 150 weeks in mixed age flocks. |
| 13 | 014 Maximum age of hens at sampling: EQUAL TO 013 Age of hens at sampling |
| | This criterion selects all records containing hens in mixed age flocks where the minimum and maximum age is the same. |
| 14 | 015 Expected depopulation date: < 1 October 2004 |
| | This criterion selects all records containing an expected depopulation date before 1 October 2004. |
| 15 | 015 Expected depopulation date: > 30 June 2007 |
| | This criterion selects all records containing an expected depopulation date after 30 June 2007. |

| Criterion No | **Rationale for the criterion** |
|---|---|
| 16 | 015 Expected depopulation date: < value of 010 Date of sampling<br><br>This criterion selects all records containing an expected depopulation before the date of sampling. |
| 17a | 016 Expected depopulation date accuracy: no and [ difference between (015 Expected depopulation date and 010 Date of sampling) > 63 ] and 011 Number of hens in flocks: >= 1000 and 014 Maximum age of hens at sampling: < 60<br><br>This criterion selects all records containing flocks of mixed age sized 1000 hens or more, with an accurate expected depopulation date, where hens are not sampled within a maximum of 9 weeks (63 days) before depopulation, and where the maximum age of hens was below the age of 60 weeks. |
| 17b | 016 Expected depopulation date accuracy: no and [ difference between (015 Expected depopulation date and 010 Date of sampling) > 63 ] and 011 Number of hens in flocks: >= 1000 and 013 Age of hens at sampling: < 60 and 014 Maximum age of hens at sampling: IS NULL<br><br>This criterion selects all records containing flocks of homogeneous age and sized 1000 hens or more, with an accurate expected depopulation date, where hens are not sampled within a maximum of 9 weeks (63 days) before depopulation, and where hens were below the age of 60 weeks. |
| 18 | 016 Expected depopulation date accuracy: no and [ difference between (015 Expected depopulation date and 010 Date of sampling) > 63 ] and 011 Number of hens in flocks: < 1000 and 013 Age of hens at sampling: < 60 and 014 Maximum age of hens at sampling: IS NULL<br><br>This criterion selects all records containing flocks of homogeneous age and sized less than 1000 hens, with an accurate expected depopulation date, where hens are not sampled within a maximum of 9 weeks (63 days) before depopulation, and where hens were below the age of 60 weeks. |
| 19 | 017 Vaccination status: no and 018 Vaccination type: IS NOT NULL<br><br>This criterion selects all records containing unvaccinated flocks with information of the type of vaccination. |
| 20 | 017 Vaccination status: unknown and 018 Vaccination type: IS NOT NULL<br><br>This criterion selects all records containing flocks with an unknown vaccination status with information of the type of vaccination. |

| Criterion No | **Rationale for the criterion** |
|---|---|
| 21 | 017 Vaccination status: no and 019 Vaccination period: IS NOT NULL<br>This criterion selects all records containing unvaccinated flocks with information of the vaccination period. |
| 22 | 017 Vaccination status: unknown and 019 Vaccination period: IS NOT NULL<br>This criterion selects all records containing flocks with an unknown vaccination status with information of the vaccination period. |
| 23 | 017 Vaccination status: no and 020 Vaccination name : IS NOT NULL<br>This criterion selects all records containing unvaccinated flocks with information of the vaccination name. |
| 24 | 017 Vaccination status: unknown and 020 Vaccination name : IS NOT NULL<br>This criterion selects all records containing flocks with an unknown vaccination status with information of the vaccination name. |
| 25 | 021 Medication status: no and 022 Medication-antimicrobial name IS NOT NULL<br>This criterion selects all records containing flocks where no antimicrobials were used during the last two weeks, with information of the antimicrobial name. |
| 26 | 026 Date of bacteriological detection testing: < 15 September 2004<br>This criterion selects all records containing a date of primary testing in the laboratory before 15 September 2004. |
| 27 | 026 Date of bacteriological detection testing: > 30 November 2005<br>This criterion selects all records containing a date of primary testing in the laboratory after November 2005. |
| 28 | 026 Date of bacteriological detection testing: < value of 010 Date of sampling<br>This criterion selects all records containing a date of primary testing in the laboratory before the date of sampling. |

| Criterion No | **Rationale for the criterion** |
|---|---|
| 29 | 027 Specimen status: no and 028 Specimen characteristics (integer or text) IS NULL and 029 Specimen characteristics comment IS NULL (EMPTY)<br>This criterion selects all records containing specimen characteristics non compliant to the technical specifications but with no information in the field 'specimen characteristics' and no information in the field 'specimen characteristic comment'. |
| 30 | 032 Reference of laboratory for serotyping: IS NULL (EMPTY) and 030 Test result is 'positive'<br>This criterion selects all records containing positive test results without information of the reference laboratory. |
| 31 | 032 Reference of laboratory for serotyping: IS NOT NULL (NOT EMPTY) and 030 Test result is 'negative'<br>This criterion selects all records containing negative test results with information of the reference laboratory. |
| 32 | 033 Isolate (*Salmonella* serovar): IS NULL (EMPTY) and 030 Test result is 'positive'<br>This criterion selects all records containing positive test results with no information of the isolate. |
| 33 | 033 Isolate (*Salmonella* serovar): IS NOT NULL (NOT EMPTY) and 030 Test result is 'negative'<br>This criterion selects all records containing negative test results with information of the isolate. |
| 34 | 014 Maximum age of hens at sampling: < 30<br>This criterion selects all records containing hens aged less than 30 weeks in mixed age flocks. |
| 35 | Difference date between: '010 Date of sampling' and '026 Date of bacteriological detection testing': > 7<br>This criterion selects all records containing a 'days to bacteriological start of test' above 7 days. |

**Step 2**

In step two holdings were selected. A criterion number 36 specified: 'Count of '023 Reference of sample': < 7'. This criterion selects all holdings with less than 7 samples.

## Annex II. Overview of the number of holdings per Member State after data validation and cleaning

| Member States | Number of holdings having at least 1000 laying hens | | | |
|---|---|---|---|---|
| | Total[1] | To be sampled[1] | Actually sampled and validated by EFSA | *Validated sampled proportion*[2] |
| | | | N | *%* |
| Austria | 769 | 362 | 337 | *43.8* |
| Belgium | 395 | 251 | 141 | *35.7* |
| Cyprus | 28 | 27 | 25 | *89.3* |
| Czech Republic | 70 | 70 | 64 | *91.4* |
| Denmark | 263 | 190 | 185 | *70.3* |
| Estonia | 11 | 11 | 11 | *100.0* |
| Finland | 558 | 307 | 250 | *44.8* |
| France | 1,840 | 518 | 511 | *27.8* |
| Germany | 2,419 | 533 | 553 | *22.9* |
| Greece | 352 | 232 | 140 | *39.8* |
| Hungary | 464 | 276 | 267 | *57.5* |
| Ireland | 180 | 172 | 146 | *81.1* |
| Italy | 1,168 | 431 | 367 | *31.4* |
| Latvia | 16 | 16 | 6 | *37.5* |
| Lithuania | 17 | 17 | 9 | *52.9* |
| Luxembourg | 9 | 9 | 9 | *100.0* |
| Poland | 1,238 | 440 | 328 | *26.5* |
| Portugal | 220 | 166 | 44 | *20.0* |
| Slovenia | 138 | 138 | 98 | *71.0* |
| Spain | 1,100 | 422 | 485 | *44.1* |
| Sweden | 303 | 210 | 168 | *55.4* |
| The Netherlands | 1,553 | 474 | 409 | *26.3* |
| The United Kingdom | 1,202 | 436 | 454 | *37.8* |
| EU[3] | 14,313 | 5,708 | 5,007 | *35.0* |
| Norway | 761 | 360 | 303 | *39.8* |

[1]: Based on Technical specifications 'Baseline study on the prevalence of Salmonella in laying flocks of Gallus gallus in the EU' In case more updated figures have been provided in the Member States' final reports on the study results, this figure has been usec

[2]: Validated sampled proportion = actually sampled and validated by EFSA / Total * 100
In the following countries a small proportion of holdings were of size less than 1000 laying hens: Czech Republic, Ireland, Luxembourg and Slovenia

[3]: These EU figures do not include data for Malta and Slovakia

## Annex III. Overview of the number of records with non-plausible characteristics in the final dataset received by the European Commission

| | Number of the exclusion criterion | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Member State** | **1** | **2** | **3** | **4** | **5** | **7** | **8** | **9** | **10** | **11** | **13** | **14** | **15** | **16** | **17a** | **17b** | **18** | **26** | **27** | **28** | **29** | **30** | **32** | **34** | **35** |
| | number of samples with non-plausible characteristics | | | | | | | | | | | | | | | | | | | | | | | |
| Austria | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 84 |
| Belgium | 0 | 0 | 0 | 0 | 42 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| Cyprus | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Czech Republic | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 9 |
| Denmark | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Estonia | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Finland | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 63 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 8 |
| France | 14 | 0 | 0 | 0 | 0 | 0 | 14 | 7 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 77 |
| Germany | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 42 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 14 |
| Greece | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 7 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 6 | 6 | 0 | 46 |
| Hungary | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 21 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| Ireland | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 23 |
| Italy | 0 | 0 | 0 | 0 | 21 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 55 |
| Latvia | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lithuania | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| Luxembourg | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 |
| Poland | 0 | 0 | 7 | 0 | 14 | 0 | 0 | 14 | 42 | 14 | 0 | 14 | 0 | 14 | 0 | 175 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 |
| Portugal | 0 | 35 | 0 | 0 | 217 | 0 | 42 | 0 | 21 | 7 | 0 | 21 | 7 | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 14 |
| SK | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 231 | 0 | 0 | 7 | 42 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 21 | 4 |
| Slovenia | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 63 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Spain | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 154 |
| Sweden | 0 | 0 | 0 | 0 | 0 | 77 | 0 | 0 | 7 | 0 | 0 | 7 | 0 | 7 | 0 | 0 | 0 | 0 | 70 | 0 | 0 | 0 | 0 | 0 | 29 |
| The Netherlands | 0 | 20 | 0 | 0 | 385 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 49 |
| The United Kingdom | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| EU | 14 | 55 | 7 | 14 | 693 | 98 | 56 | 35 | 217 | 21 | 231 | 56 | 7 | 147 | 42 | 210 | 21 | 3 | 71 | 27 | 0 | 7 | 7 | 28 | 590 |
| Norway | 0 | 0 | 0 | 0 | 21 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 53 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |

**Annex IV. Observed prevalence of *Salmonella* Enteritidis, of *Salmonella* Typhimurium and of serovars other than *Salmonella* Enteritidis and *Salmonella* Typhimurium in holdings of laying hens in the EU, 2004-**

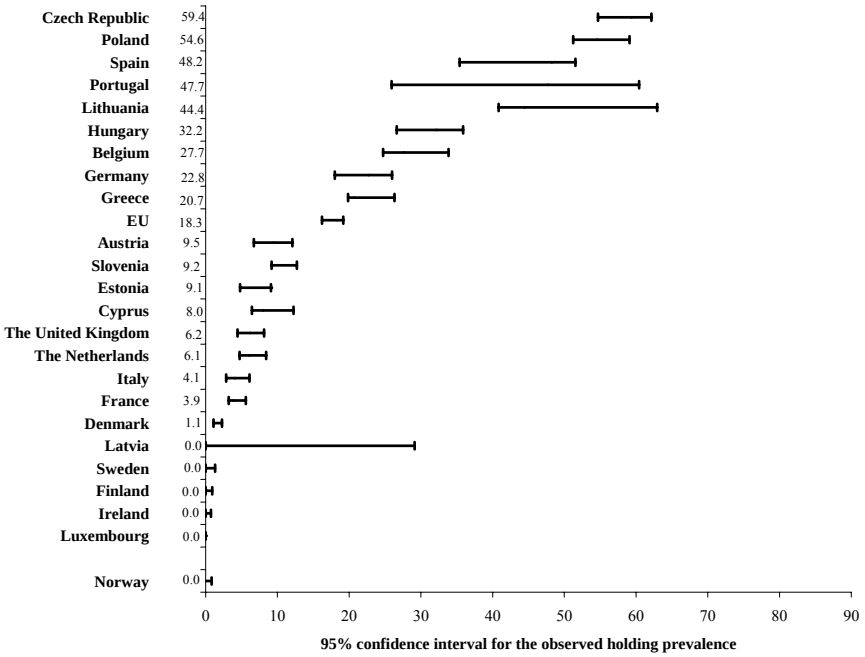
| | | *S.* Enteritidis | | *S.* Typhimurium | | *S.* non-Enteritidis/Typhimurium | |
|---|---|---|---|---|---|---|---|
| | **N** | **% pos** | **CI 95%[a]** | **% pos** | **CI 95%** | **% pos** | **CI 95%** |
| Austria | 337 | 9.5 | 7.4 - 12.1 | 1.2 | 0.5 - 2.4 | 5.3 | 3.8 - 7.4 |
| Belgium | 141 | 27.7 | 22.1 - 33.9 | 0.7 | 0.0 - 3.0 | 19.9 | 15.1 - 25.6 |
| Cyprus | 25 | 8.0 | 3.7 - 12.3 | 0.0 | 0.0 - 2.9 | 24.0 | 18.1 - 28.5 |
| Czech Republic | 64 | 59.4 | 54.9 - 62.1 | 4.7 | 2.9 - 6.6 | 7.8 | 5.8 - 9.9 |
| Denmark | 185 | 1.1 | 0.4 - 2.3 | 0.5 | 0.0 - 1.6 | 1.1 | 0.4 - 2.3 |
| Estonia | 11 | 9.1 | -[b] | 0.0 | - | 9.1 | - |
| Finland | 250 | 0.0 | 0.0 - 0.9 | 0.4 | 0.0 - 1.6 | 0.0 | 0.0 - 0.9 |
| France | 511 | 3.9 | 2.7 - 5.6 | 4.3 | 3.0 - 6.1 | 10.4 | 8.3 - 12.8 |
| Germany | 553 | 22.8 | 19.9 - 26.0 | 2.0 | 1.2 - 3.3 | 14.8 | 12.4 - 17.6 |
| Greece | 140 | 20.7 | 15.9 - 26.3 | 6.4 | 3.9 - 10.4 | 35.7 | 29.7 - 42.0 |
| Hungary | 267 | 32.2 | 28.6 - 35.9 | 2.6 | 1.6 - 4.2 | 16.1 | 13.4 - 19.1 |
| Ireland | 146 | 0.0 | 0.0 - 0.7 | 0.0 | 0.0 - 0.7 | 1.4 | 0.6 - 2.6 |
| Italy | 367 | 4.1 | 2.7 - 6.1 | 3.8 | 2.5 - 5.8 | 23.7 | 20.3 - 27.5 |
| Latvia | 6 | 0.0 | 0.0 - 29.1 | 0.0 | 0.0 - 29.1 | 16.7 | 1.0 - 46.8 |
| Lithuania | 9 | 44.4 | 22.6 - 62.9 | 0.0 | 0.0 - 17.4 | 11.1 | 0.5 - 31.1 |
| Luxembourg | 9 | 0.0 | - | 0.0 | - | 0.0 | - |
| Poland | 328 | 54.6 | 49.9 - 59.1 | 2.1 | 1.1 - 3.9 | 34.5 | 30.2 - 38.9 |
| Portugal | 44 | 47.7 | 34.9 - 60.4 | 4.5 | 1.3 - 13.7 | 56.8 | 43.4 - 68.8 |
| Slovenia | 98 | 9.2 | 6.4 - 12.7 | 0.0 | 0.0 - 1.4 | 10.2 | 7.3 - 13.8 |
| Spain | 485 | 48.2 | 44.9 - 51.5 | 5.4 | 4.0 - 7.1 | 48.2 | 44.9 - 51.5 |
| Sweden | 168 | 0.0 | 0.0 - 1.3 | 0.0 | 0.0 - 1.3 | 0.0 | 0.0 - 1.3 |
| The Netherlands | 409 | 6.1 | 4.4 - 8.4 | 1.7 | 0.9 - 3.2 | 8.6 | 6.5 - 11.2 |
| The United Kingdom | 454 | 6.2 | 4.6 - 8.2 | 1.8 | 1.0 - 3.0 | 4.6 | 3.3 - 6.4 |
| EU[b] | 5,007 | 17.8 | | 2.5 | | 16.3 | |
| **EU weighted prevalence** | | **18.3** | **17.5 - 19.2** | **2.6** | **2.2 - 3.0** | **17.1** | **16.3 - 18.0** |
| Norway | 303 | 0.0 | 0.0 - 0.8 | 0.0 | 0.0 - 0.8 | 0.0 | 0.0 - 0.8 |

[a]: Confidence interval

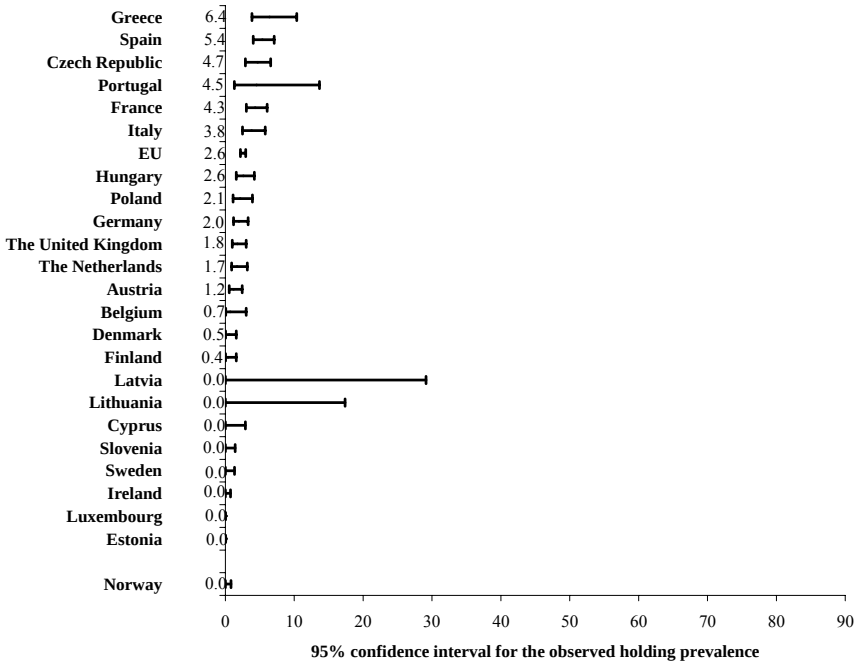
[b]: No confidence interval for Estonia and Luxembourg since all holdings in these MSs were sampled

[c]: These EU figures do not include data for Malta and Slovakia

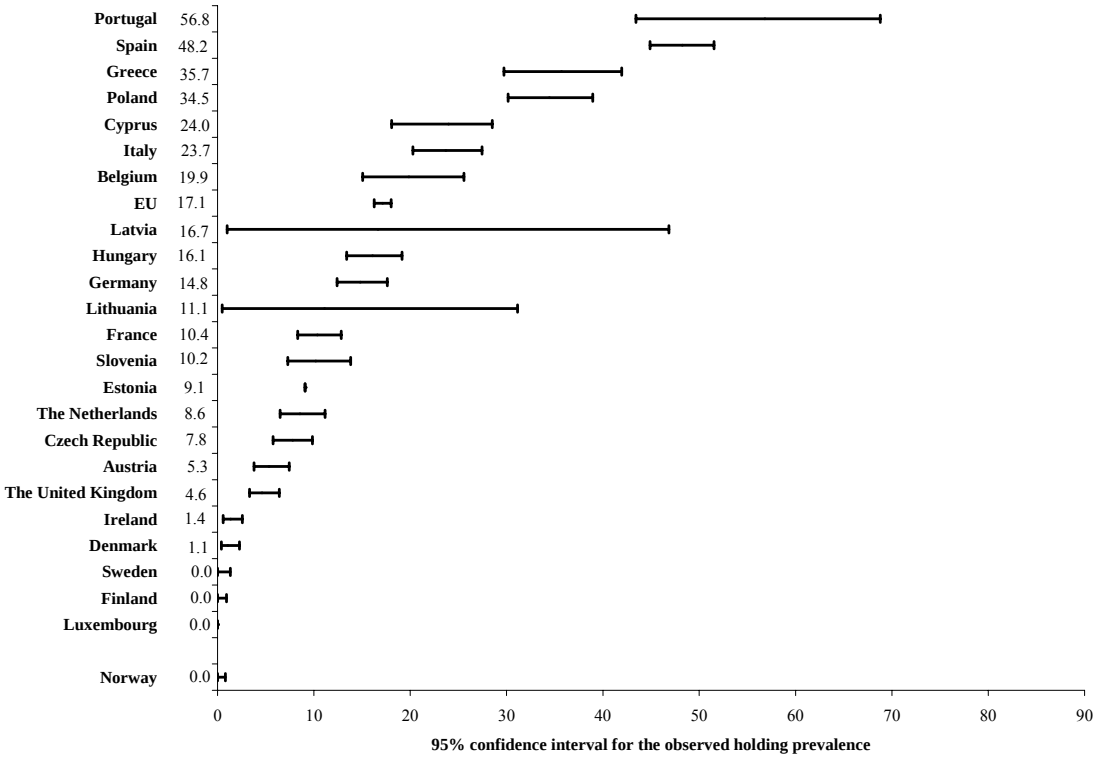
## Annex V. Observed prevalence of *Salmonella* Enteritidis-positive holdings with 95% confidence intervals in the EU, 2004-2005



## Annex VI. Observed prevalence of *Salmonella* Typhimurium-positive holdings with 95% confidence intervals in the EU, 2004-2005



**Annex VII. Observed prevalence of holdings positive for serovars other than *Salmonella* Enteritidis and *Salmonella* Typhimurium with 95% confidence intervals in the EU, 2004-2005**



**Annex VIII. Frequency distribution of *Salmonella* serovars in laying hen holdings in EU MSs, 2004-2005**

The countries that are not included in this overview are first those that did not isolate any *Salmonella* (Luxembourg, Sweden and Norway) and those that had no data included in the validated database (Malta and Slovakia).

| **Austria** | **Serovars (N=195)** | | | **Holdings with serovars (N=52)** | |
|---|---|---|---|---|---|
| | | **N** | **%** | **N** | **%** |
| | S. Enteritidis | 130 | 66.7 | 32 | 61.5 |
| | S. Infantis | 15 | 7.7 | 4 | 7.7 |
| | S. Typhimurium | 12 | 6.2 | 4 | 7.7 |
| | S. Montevideo | 11 | 5.6 | 3 | 5.8 |
| | S. Tennessee | 9 | 4.6 | 3 | 5.8 |
| | S. Mbandaka | 7 | 3.6 | 2 | 3.8 |
| | S. Braenderup | 5 | 2.6 | 2 | 3.8 |
| | *Salmonella* subsp. diarizonae | 2 | 1.0 | 2 | 3.8 |
| | S. Senftenberg | 2 | 1.0 | 2 | 3.8 |
| | S. Bredeney | 1 | 0.5 | 1 | 1.9 |
| | S. Agona | 1 | 0.5 | 1 | 1.9 |
| | Other serovars | 0 | 0.0 | | |
| | *S.* non typeable | 0 | 0.0 | | |

| **Belgium** | **Serovars (N=202)** | | | **Holdings with serovars (N=53)** | |
|---|---|---|---|---|---|
| | | **N** | **%** | **N** | **%** |
| | S. Enteritidis | 142 | 70.3 | 39 | 73.6 |
| | S. Mbandaka | 11 | 5.4 | 5 | 9.4 |
| | S. Senftenberg | 7 | 3.5 | 5 | 9.4 |
| | S. Braenderup | 10 | 5.0 | 3 | 5.7 |
| | S. Agona | 6 | 3.0 | 3 | 5.7 |
| | S. Rissen | 4 | 2.0 | 3 | 5.7 |
| | S. Infantis | 2 | 1.0 | 2 | 3.8 |
| | S. Livingstone | 4 | 2.0 | 1 | 1.9 |
| | S. Montevideo | 3 | 1.5 | 1 | 1.9 |
| | S. Indiana | 2 | 1.0 | 1 | 1.9 |
| | Other serovars | 5 | 2.5 | | |
| | *S.* non typeable | 6 | 3.0 | 6 | 11.3 |

| Cyprus | Serovars (N=22) | | Holdings with serovars (N=7) | |
|---|---|---|---|---|
| | N | % | N | % |
| *S.* Enteritidis | 2 | 9.1 | 2 | 28.6 |
| *S.* Newport | 4 | 18.2 | 1 | 14.3 |
| *S.* Virchow | 4 | 18.2 | 1 | 14.3 |
| *S.* Brandenburg | 3 | 13.6 | 1 | 14.3 |
| *S.* Muenchen | 2 | 9.1 | 1 | 14.3 |
| *S.* Naga | 2 | 9.1 | 1 | 14.3 |
| *S.* Braenderup | 1 | 4.5 | 1 | 14.3 |
| Other serovars | 0 | 0.0 | | |
| *S.* non typeable | 4 | 18.2 | 1 | 14.3 |

| Czech Republic | Serovars (N=153) | | Holdings with serovars (N=42) | |
|---|---|---|---|---|
| | N | % | N | % |
| *S.* Enteritidis | 132 | 86.3 | 38 | 90.5 |
| *S.* Typhimurium | 8 | 5.2 | 3 | 7.1 |
| *S.* Infantis | 4 | 2.6 | 2 | 4.8 |
| *S.* Saintpaul | 7 | 4.6 | 1 | 2.4 |
| *S.* Schwarzengrund | 1 | 0.7 | 1 | 2.4 |
| *S.* Lille | 1 | 0.7 | 1 | 2.4 |
| Other serovars | 0 | 0.0 | | |
| *S.* non typeable | 0 | 0.0 | | |

| Denmark | Serovars (N=14) | | Holdings with serovars (N=5) | |
|---|---|---|---|---|
| | N | % | N | % |
| *S.* Infantis | 7 | 50.0 | 2 | 40.0 |
| *S.* Enteritidis | 6 | 42.9 | 2 | 40.0 |
| *S.* Typhimurium | 1 | 7.1 | 1 | 20.0 |
| Other serovars | 0 | 0.0 | | |
| *S.* non typeable | 0 | 0.0 | | |

| Estonia | Serovars (N=3) | | Holdings with serovars (N=2) | |
|---|---|---|---|---|
| | N | % | N | % |
| *S.* Enteritidis | 2 | 66.7 | 1 | 50.0 |
| *S.* Isangi | 1 | 33.3 | 1 | 50.0 |
| Other serovars | 0 | 0.0 | | |
| *S.* non typeable | 0 | 0.0 | | |

| Finland | Serovars (N=1) | | Holdings with serovars (N=1) | |
|---|---|---|---|---|
| | N | % | N | % |
| S. Typhimurium | 1 | 100.0 | 1 | 100.0 |
| Other serovars | 0 | 0.0 | | |
| *S.* non typeable | 0 | 0.0 | | |

| France | Serovars (N=273) | | Holdings with serovars (N=88) | |
|---|---|---|---|---|
| | N | % | N | % |
| S. Typhimurium | 55 | 20.1 | 22 | 25.0 |
| S. Enteritidis | 64 | 23.4 | 20 | 22.7 |
| S. Infantis | 24 | 8.8 | 8 | 9.1 |
| S. Mbandaka | 14 | 5.1 | 7 | 8.0 |
| S. Anatum | 8 | 2.9 | 6 | 6.8 |
| S. Tennessee | 15 | 5.5 | 5 | 5.7 |
| S. Braenderup | 14 | 5.1 | 5 | 5.7 |
| S. Livingstone | 13 | 4.8 | 5 | 5.7 |
| S. Montevideo | 14 | 5.1 | 3 | 3.4 |
| S. Virchow | 13 | 4.8 | 3 | 3.4 |
| Other serovars | 39 | 14.3 | | |
| *S.* non typeable | 0 | 0.0 | | |

| Germany | Serovars (N=550) | | Holdings with serovars (N=160) | |
|---|---|---|---|---|
| | N | % | N | % |
| S. Enteritidis | 353 | 64.2 | 126 | 78.8 |
| *S.* subsp. enterica rough | 99 | 18.0 | 48 | 30.0 |
| S. Typhimurium | 29 | 5.3 | 11 | 6.9 |
| S. Infantis | 22 | 4.0 | 9 | 5.6 |
| S. Mbandaka | 5 | 0.9 | 4 | 2.5 |
| S. Livingstone | 10 | 1.8 | 3 | 1.9 |
| S. Rissen | 5 | 0.9 | 3 | 1.9 |
| S. Tennessee | 3 | 0.5 | 3 | 1.9 |
| *S.* Group E | 3 | 0.5 | 3 | 1.9 |
| S. Hadar | 4 | 0.7 | 2 | 1.3 |
| *S.* Group D | 5 | 0.9 | 1 | 0.6 |
| Other serovars | 12 | 2.2 | | |
| *S.* non typeable | 0 | 0.0 | | |

| Greece | Serovars (N=244) | | | Holdings with serovars (N=69) | |
|---|---|---|---|---|---|
| | | N | % | N | % |
| | S. Enteritidis | 80 | 32.8 | 29 | 42.0 |
| | S. Infantis | 18 | 7.4 | 9 | 13.0 |
| | S. Typhimurium | 16 | 6.6 | 9 | 13.0 |
| | S. Livingstone | 23 | 9.4 | 8 | 11.6 |
| | S. Braenderup | 16 | 6.6 | 7 | 10.1 |
| | S. Virchow | 9 | 3.7 | 3 | 4.3 |
| | S. Agona | 4 | 1.6 | 2 | 2.9 |
| | S. Bredeney | 3 | 1.2 | 2 | 2.9 |
| | S. Rissen | 7 | 2.9 | 1 | 1.4 |
| | S. Isangi | 4 | 1.6 | 1 | 1.4 |
| | S. Poona | 3 | 1.2 | 1 | 1.4 |
| | Other serovars | 9 | 3.7 | | |
| | *S.* non typeable | 52 | 21.3 | | |

| Hungary | Serovars (N=389) | | | Holdings with serovars (N=117) | |
|---|---|---|---|---|---|
| | | N | % | N | % |
| | S. Enteritidis | 282 | 72.5 | 86 | 73.5 |
| | S. Infantis | 17 | 4.4 | 11 | 9.4 |
| | S. Bovismorbificans | 14 | 3.6 | 4 | 3.4 |
| | S. Mbandaka | 14 | 3.6 | 5 | 4.3 |
| | S. Typhimurium | 13 | 3.3 | 7 | 6.0 |
| | S. Agona | 11 | 2.8 | 4 | 3.4 |
| | S. Bredeney | 10 | 2.6 | 4 | 3.4 |
| | *S.* Group D | 5 | 1.3 | 1 | 0.9 |
| | S. Blockley | 3 | 0.8 | 2 | 1.7 |
| | S. Give | 3 | 0.8 | 1 | 0.9 |
| | S. Senftenberg | 3 | 0.8 | 3 | 2.6 |
| | Other serovars | 14 | 3.6 | | |
| | *S.* non typeable | 0 | 0.0 | | |

| Ireland | Serovars (N=2) | | | Holdings with serovars (N=2) | |
|---|---|---|---|---|---|
| | | N | % | N | % |
| | *S.* Reading | 1 | 50.0 | 1 | 50.0 |
| | *S.* Brandenburg | 1 | 50.0 | 1 | 50.0 |
| | Other serovars | 0 | 0.0 | | |
| | *S.* non typeable | 0 | 0.0 | | |

| Italy | Serovars (N=357) | | Holdings with serovars (N=107) | |
|---|---|---|---|---|
| | N | % | N | % |
| S. Enteritidis | 41 | 11.5 | 15 | 14.0 |
| S. Hadar | 40 | 11.2 | 14 | 13.1 |
| S. Typhimurium | 27 | 7.6 | 14 | 13.1 |
| S. Infantis | 18 | 5.0 | 12 | 11.2 |
| S. Bredeney | 28 | 7.8 | 11 | 10.3 |
| S. Mbandaka | 19 | 5.3 | 8 | 7.5 |
| S. Kentucky | 10 | 2.8 | 6 | 5.6 |
| S. Livingstone | 18 | 5.0 | 5 | 4.7 |
| S. Thompson | 12 | 3.4 | 5 | 4.7 |
| S. Braenderup | 11 | 3.1 | 5 | 4.7 |
| S. Virchow | 10 | 2.8 | 5 | 4.7 |
| Other serovars | 117 | 32.8 | | |
| *S.* non typeable | 6 | 1.7 | | |

| Latvia | Serovars (N=1) | | Holdings with serovars (N=1) | |
|---|---|---|---|---|
| | N | % | N | % |
| *S* . Santemarie | 1 | 100.0 | 1 | 100.0 |
| Other serovars | 0 | 0.0 | | |
| *S.* non typeable | 0 | 0.0 | | |

| Lithuania | Serovars (N=9) | | Holdings with serovars (N=4) | |
|---|---|---|---|---|
| | N | % | N | % |
| S. Enteritidis | 8 | 88.9 | 4 | 100.0 |
| Other serovars | 0 | 0.0 | 0 | 0.0 |
| *S.* non typeable | 1 | 11.1 | 1 | 25.0 |

| Poland | Serovars (N=1,092) | | | Holdings with serovars (N=250) | |
|---|---|---|---|---|---|
| | | N | % | N | % |
| | S. Enteritidis | 665 | 60.9 | 179 | 71.6 |
| | S. Infantis | 198 | 18.1 | 54 | 21.6 |
| | S. Mbandaka | 67 | 6.1 | 20 | 8.0 |
| | S. Virchow | 59 | 5.4 | 14 | 5.6 |
| | S. Hadar | 24 | 2.2 | 10 | 4.0 |
| | S. Typhimurium | 12 | 1.1 | 7 | 2.8 |
| | S. Anatum | 9 | 0.8 | 4 | 1.6 |
| | S. Indiana | 8 | 0.7 | 4 | 1.6 |
| | S. Livingstone | 12 | 1.1 | 3 | 1.2 |
| | S. Schwarzengrund | 8 | 0.7 | 2 | 0.8 |
| | Other serovars | 28 | 2.6 | | |
| | *S.* non typeable | 2 | 0.2 | | |

| Portugal | Serovars (N=145) | | | Holdings with serovars (N=35) | |
|---|---|---|---|---|---|
| | | N | % | N | % |
| | S. Enteritidis | 59 | 40.7 | 21 | 60.0 |
| | S. Mbandaka | 49 | 33.8 | 17 | 48.6 |
| | S. Tennessee | 6 | 4.1 | 5 | 14.3 |
| | S. Heidelberg | 12 | 8.3 | 2 | 5.7 |
| | S. Agona | 5 | 3.4 | 2 | 5.7 |
| | S. Typhimurium | 4 | 2.8 | 2 | 5.7 |
| | *S.* rough | 1 | 0.7 | 1 | 2.9 |
| | *S.* partly typable | 1 | 0.7 | 1 | 2.9 |
| | S. Rissen | 1 | 0.7 | 1 | 2.9 |
| | S. Livingstone | 1 | 0.7 | 1 | 2.9 |
| | S. Havana | 1 | 0.7 | 1 | 2.9 |
| | S. Hadar | 1 | 0.7 | 1 | 2.9 |
| | S. Give | 1 | 0.7 | 1 | 2.9 |
| | Other serovars | 0 | 0.0 | | |
| | *S.* non typeable | 3 | 2.1 | | |

| Slovenia | Serovars (N=65) | | | Holdings with serovars (N=19) | |
|---|---|---|---|---|---|
| | | N | % | N | % |
| | *S.* Enteritidis | 44 | 67.7 | 9 | 47.4 |
| | *S.* Infantis | 5 | 7.7 | 2 | 10.5 |
| | *S.* Rissen | 4 | 6.2 | 2 | 10.5 |
| | *S.* Agona | 3 | 4.6 | 2 | 10.5 |
| | *S.* Menden | 5 | 7.7 | 1 | 5.3 |
| | *S.* Stanleyville | 2 | 3.1 | 1 | 5.3 |
| | *S.* Mbandaka | 1 | 1.5 | 1 | 5.3 |
| | *S.* Heidelberg | 1 | 1.5 | 1 | 5.3 |
| | Other serovars | 0 | 0.0 | | |
| | *S.* non typeable | 0 | 0.0 | | |

| Spain | Serovars (N=1,601) | | | Holdings with serovars (N=355) | |
|---|---|---|---|---|---|
| | | N | % | N | % |
| | *S.* Enteritidis | 758 | 47.3 | 234 | 65.9 |
| | *S.* Infantis | 149 | 9.3 | 55 | 15.5 |
| | *S.* Ohio | 102 | 6.4 | 34 | 9.6 |
| | *S.* Typhimurium | 59 | 3.7 | 26 | 7.3 |
| | *S.* Mbandaka | 36 | 2.2 | 24 | 6.8 |
| | *S.* Hadar | 47 | 2.9 | 21 | 5.9 |
| | *S.* Livingstone | 58 | 3.6 | 19 | 5.4 |
| | *S.* Virchow | 33 | 2.1 | 11 | 3.1 |
| | *S.* Montevideo | 27 | 1.7 | 11 | 3.1 |
| | *S.* Altona | 21 | 1.3 | 9 | 2.5 |
| | *S.* Bredeney | 21 | 1.3 | 8 | 2.3 |
| | Other serovars | 289 | 18.1 | | |
| | *S.* non typeable | 1 | 0.1 | | |

| The Netherlands | Serovars (N=202) | | | Holdings with serovars (N=63) | |
|---|---|---|---|---|---|
| | | N | % | N | % |
| | *S.* Enteritidis | 98 | 48.5 | 25 | 39.7 |
| | *S.* Typhimurium | 13 | 6.4 | 7 | 11.1 |
| | *S.* Senftenberg | 11 | 5.4 | 6 | 9.5 |
| | *S.* Agona | 10 | 5.0 | 5 | 7.9 |
| | *S.* Livingstone | 10 | 5.0 | 3 | 4.8 |
| | *S.* Virchow | 10 | 5.0 | 3 | 4.8 |
| | *S.* Duisburg | 8 | 4.0 | 3 | 4.8 |
| | *S.* Montevideo | 5 | 2.5 | 2 | 3.2 |
| | *S.* Braenderup | 7 | 3.5 | 1 | 1.6 |
| | *S.* Paratyphi B/Paratyphi B var. Java | 5 | 2.5 | 1 | 1.6 |
| | Other serovars | 25 | 12.4 | | |
| | *S.* non typeable | 0 | 0.0 | | |

| The United Kingdom | Serovars (N=178) | | | Holdings with serovars (N=54) | |
|---|---|---|---|---|---|
| | | N | % | N | % |
| | *S.* Enteritidis | 114 | 64.0 | 28 | 51.9 |
| | *S.* Typhimurium | 22 | 12.4 | 8 | 14.8 |
| | *S.* Mbandaka | 7 | 3.9 | 4 | 7.4 |
| | *S.* Livingstone | 6 | 3.4 | 2 | 3.7 |
| | *S.* Senftenberg | 5 | 2.8 | 3 | 5.6 |
| | *S.* Thompson | 3 | 1.7 | 1 | 1.9 |
| | *S.* Virchow | 2 | 1.1 | 1 | 1.9 |
| | *S.* Tennessee | 2 | 1.1 | 1 | 1.9 |
| | *S.* Yoruba | 2 | 1.1 | 2 | 3.7 |
| | *S.* Infantis | 2 | 1.1 | 1 | 1.9 |
| | Other serovars | 13 | 7.3 | | |
| | *S.* non typeable | 0 | 0.0 | | |

## Annex IX. Bivariate description of *Salmonella*-positive samples and of *Salmonella*-positive holdings for the investigated factors

### *Salmonella* Enteritidis

MS-Group 1 contains those Member states that had a *S.* Enteritidis observed holding prevalence below 2.5% (Denmark, Finland, Ireland, Latvia, Luxembourg and Sweden)

MS-Group 2 contains those Member states that had a *S.* Enteritidis observed holding prevalence between 2.5% and 15% (Austria, Cyprus, Estonia, France, Italy, Slovenia, the Netherlands and the United Kingdom)

MS-Group 3 contains those Member states that had a *S.* Enteritidis observed holding prevalence above 15% (Belgium, the Czech Republic, Germany, Greece, Hungary, Lithuania, Poland, Portugal and Spain)

| Factor | EU | | | MS-group 1 | | | MS-group 2 | | | MS-group 3 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N obs | % pos | 95% CI | N obs | % pos | 95% CI | N obs | % pos | 95% CI | N obs | % pos | 95% CI |
| **Overall** | 26,656 | 8.9 | 8.3 ; 9.5 | 4,081 | 0.1 | 0.0 ; 0.2 | 10,731 | 2.8 | 2 | 11,844 | 16.9 | 15.8 ; 18.1 |
| **Sample-level** | | | | | | | | | | | | |
| **Sample type** | | | | | | | | | | | | |
| Faeces | 19,040 | 8.5 | 7.9 ; 9.1 | 2,915 | 0.1 | 0 ; 0.2 | 7,665 | 2.6 | 2 ; 3.2 | 8,460 | 16.2 | 15 ; 17.4 |
| Dust | 7,616 | 10.1 | 9.3 ; 10.8 | 1,666 | 0.1 | 0 ; 0.3 | 3,066 | 3.4 | 2.7 ; 4.2 | 3,384 | 18.8 | 17.3 ; 20.3 |
| **Overall** | 3,808 | 19.1 | 18 ; 20.1 | 583 | 0.3 | 0.1 ; 0.5 | 1,533 | 5.6 | 4.5 ; 6.5 | 1,692 | 36.5 | 34.4 ; 38.8 |
| **Flock-level** | | | | | | | | | | | | |
| **Flock production type** | | | | | | | | | | | | |
| cage | 2,303 | 24.9 | 23.3 ; 26.4 | 314 | 0.5 | 0.1 ; 0.9 | 673 | 8.1 | 6.3 ; 9.9 | 1,316 | 39.1 | 36.6 ; 41.5 |
| barn | 600 | 18.8 | 15.9 ; 21.8 | 83 | 0 | - | 249 | 4.5 | 2.2 ; 6.8 | 268 | 33.6 | 28.3 ; 38.9 |
| free-range standard | 633 | 5.0 | 3.3 ; 6.8 | 105 | 0 | - | 458 | 3.1 | 1.7 ; 4.5 | 70 | 18.3 | 9.0 ; 27.6 |
| organic | 272 | 8.5 | 4.8 ; 12.2 | 81 | 0 | - | 153 | 3.5 | 0.8 ; 6.2 | 38 | 31.8 | 17.0 ; 46.7 |
| **Age type flock** | | | | | | | | | | | | |
| Homogeneous age | 3,670 | 19.1 | 18.1 ; 20.2 | 575 | 0.3 | 0.1 ; 0.5 | 1,487 | 5.5 | 4.5 ; 6.5 | 1,608 | 37.5 | 35.3 ; 39.7 |
| Mixed age | 138 | 16.6 | 10.9 ; 22.3 | 8 | 0 | - | 46 | 6.1 | 0.0 ; 12.4 | 84 | 21.9 | 14 ; 29.9 |

| Factor | EU | | | MS-group 1 | | | MS-group 2 | | | MS-group 3 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N obs | % pos | 95% CI | N obs | % pos | 95% CI | N obs | % pos | 95% CI | N obs | % pos | 95% CI |
| **Overall** | 3,808 | 19.1 | 18 ; 20.1 | 583 | 0.3 | 0.1 ; 0.5 | 1,533 | 5.6 | 4.5 ; 6.5 | 1,692 | 36.5 | 34.4 ; 38.8 |
| **Flock-level** | | | | | | | | | | | | |
| **Age of hens at sampling (weeks)** | | | | | | | | | | | | |
| Q1 | | | | | | | | | | | | |
| EU: (26, 61)[a] | | | | | | | | | | | | |
| Grp 1: (30, 64) | 970 | 17.1 | 15 ; 19.2 | 173 | 0 | - | 421 | 5.2 | 3.3 ; 7 | 447 | 29.5 | 25.5 ; 33.6 |
| Grp 2: (30, 62) | | | | | | | | | | | | |
| Grp 3: (26, 58) | | | | | | | | | | | | |
| Q2 | | | | | | | | | | | | |
| EU: (>61, 68) | | | | | | | | | | | | |
| Grp 1: (>64, 68) | 997 | 12.2 | 10.3 ; 14.2 | 148 | 0 | - | 430 | 4.8 | 3 ; 6.6 | 446 | 31.7 | 27.5 ; 35.9 |
| Grp 2: (>62, 68) | | | | | | | | | | | | |
| Grp 3: (>58, 70) | | | | | | | | | | | | |
| Q3 | | | | | | | | | | | | |
| EU: (>68, 75) | | | | | | | | | | | | |
| Grp 1: (>68, 71) | 944 | 16.4 | 14.1 ; 18.7 | 125 | 0.7 | 0 ; 1.5 | 321 | 4.3 | 2.4 ; 6.2 | 409 | 40 | 35.5 ; 44.6 |
| Grp 2: (>68, 73) | | | | | | | | | | | | |
| Grp 3: (>70, 80) | | | | | | | | | | | | |
| >Q3 | | | | | | | | | | | | |
| EU: (>75, 150) | | | | | | | | | | | | |
| Grp 1: (>71, 104) | 897 | 31.2 | 28.6 ; 33.9 | 137 | 0.6 | 0 ; 1.4 | 361 | 7.9 | 5.4 ; 10.4 | 390 | 46.3 | 41.7 ; 50.9 |
| Grp 2: (>73, 150) | | | | | | | | | | | | |
| Grp 3: (>80, 150) | | | | | | | | | | | | |
| **Vaccination status** | | | | | | | | | | | | |
| Unvaccinated | 2,190 | 22.8 | 21.4 ; 24.2 | 583 | 0.3 | 0.1 ; 0.5 | 707 | 6.9 | 5.2 ; 8.6 | 900 | 50.2 | 47.3 ; 53 |
| Vacc. with SE vaccine | 1,095 | 12.1 | 10.3 ; 14 | - | - | - | 649 | 4.3 | 2.9 ; 5.7 | 446 | 23.6 | 19.6 ; 27.6 |
| Vacc. with non-SE vaccine | 523 | 21.9 | 18.5 ; 25.3 | - | - | - | 177 | 4.3 | 1.6 ; 6.8 | 346 | 29.7 | 25 ; 34.3 |
| **Medication status** | | | | | | | | | | | | |
| No antimicrobials used during the last two weeks | 3,768 | 19.1 | 18 ; 20.1 | 583 | 0.3 | 0.1 ; 0.5 | 1,506 | 5.6 | 4.5 ; 6.6 | 1,679 | 36.4 | 34.3 ; 38.3 |
| Antimicrobials used during the last two weeks | 40 | 18.2 | 6.8 ; 29.6 | - | - | - | 27 | 3.2 | 0 ; 8.7 | 13 | 52 | 24.2 ; 79.8 |

Q1, Q2, and Q3: Quartiles 1 to 3 (The 'Quartiles 1 to 3' are the lower, middle and upper quartiles and they divide the data in categories with, respectively, all values being smaller than 25%, 50% and 75% of the remaining data)

[a] Minimum, maximum category value.

| Factor | EU | | | MS-group 1 | | | MS-group 2 | | | MS-group 3 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N obs | % pos | 95% CI | N obs | % pos | 95% CI | N obs | % pos | 95% CI | N obs | % pos | 95% CI |
| **Overall** | 3,808 | 19.1 | 18 ; 20.1 | 583 | 0.3 | 0.1 ; 0.5 | 1,533 | 5.6 | 4.5 ; 6.5 | 1,692 | 36.5 | 34.4 ; 38.8 |
| **Holding-level** | | | | | | | | | | | | |
| **Number of hens in holding** | | | | | | | | | | | | |
| Q1 | | | | | | | | | | | | |
| EU: (30; 3,000) | | | | | | | | | | | | |
| Grp 1: (61; 2,250) | 997 | 8.7 | 7 ; 10.4 | 146 | 0 | - | 387 | 3.2 | 1.7 ; 4.7 | 423 | 16.5 | 13.2 ; 19.8 |
| Grp 2: (30; 2,900) | | | | | | | | | | | | |
| Grp 3: (200; 3,791) | | | | | | | | | | | | |
| Q2 | | | | | | | | | | | | |
| EU: (>3,000; 8,000) | | | | | | | | | | | | |
| Grp 1: (>2,250; 4,500) | 938 | 12.8 | 10.7 ; 14.8 | 146 | 0 | - | 380 | 3.8 | 2.1 ; 5.4 | 425 | 34.3 | 29.9 ; 38.7 |
| Grp 2: (>2,900; 6,790) | | | | | | | | | | | | |
| Grp 3: (>,3791; 11,800) | | | | | | | | | | | | |
| Q3 | | | | | | | | | | | | |
| EU: (>8,000; 23,000) | | | | | | | | | | | | |
| Grp 1: (>4,500; 9,000) | 925 | 21.1 | 18.6 ; 23.5 | 147 | 0 | - | 385 | 4.8 | 3 ; 6.7 | 421 | 44.3 | 39.8 ; 48.7 |
| Grp 2: (>6,790; 20,000) | | | | | | | | | | | | |
| Grp 3: (>11,800; 33,650) | | | | | | | | | | | | |
| >Q3 | | | | | | | | | | | | |
| EU: (>23,000; 1,200,000) | | | | | | | | | | | | |
| Grp 1: (>9,000; 150,472) | 948 | 34.1 | 31.4 ; 36.7 | 144 | 1.2 | 0.2 ; 2.1 | | 9.9 | 7.2 ; 12.6 | 423 | 57.5 | 53 ; 62 |
| Grp 2: (19,001; 850,000) | | | | | | | | | | | | |
| Grp 3: (>33,650; 1,200,000) | | | | | | | | | | | | |

| Factor | EU | | | MS-group 1 | | | MS-group 2 | | | MS-group 3 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N obs | % pos | 95% CI | N obs | % pos | 95% CI | N obs | % pos | 95% CI | N obs | % pos | 95% CI |
| **Overall** | 3,808 | 19.1 | 18 ; 20.1 | 583 | 0.3 | 0.1 ; 0.5 | 1,533 | 5.6 | 4.5 ; 6.5 | 1,692 | 36.5 | 34.4 ; 38.8 |
| **General** | | | | | | | | | | | | |
| Days to bacteriological analysis | | | | | | | | | | | | |
| 0-1 | 2,131 | 18.2 | 16.6 ; 19.7 | 437 | 0.4 | 0.1 ; 0.7 | 846 | 5.6 | 4.2 ; 7 | 848 | 34 | 31 ; 37 |
| 2 | 545 | 22.4 | 18.9 ; 25.8 | 106 | 0 | - | 183 | 6.1 | 3 ; 9.2 | 256 | 37.8 | 32 ; 43.5 |
| 3 | 517 | 28.2 | 25.2 ; 31.2 | 13 | 0 | - | 130 | 5.2 | 1.8 ; 8.5 | 374 | 43.8 | 39.8 ; 47.9 |
| 4-7 | 615 | 13.6 | 11.5 ; 15.7 | 27 | 0 | - | 374 | 5.3 | 3.3 ; 7.2 | 214 | 39.2 | 33.6 ; 44.8 |
| Months of sampling | | | | | | | | | | | | |
| January | 267 | 18.5 | 14.4 ; 22.7 | 34 | 0 | - | 124 | 9.2 | 4.9 ; 13.6 | 109 | 36.1 | 27.6 ; 44.5 |
| February | 259 | 25.3 | 20.2 ; 30.4 | 40 | 0 | - | 88 | 8.2 | 2.9 ; 13.5 | 131 | 43.7 | 35.5 ; 52 |
| March | 355 | 18.3 | 14.5 ; 22.2 | 63 | 0 | - | 148 | 5.5 | 2.2 ; 8.7 | 144 | 37.1 | 29.5 ; 44.8 |
| April | 407 | 17.8 | 14.3 ; 21.3 | 52 | 0 | - | 160 | 4.1 | 1.6 ; 6.5 | 195 | 33.5 | 27.1 ; 39.9 |
| May | 362 | 18.6 | 14.7 ; 22.4 | 45 | 0 | - | 145 | 6.2 | 2.7 ; 9.7 | 172 | 32.8 | 25.9 ; 39.6 |
| June | 372 | 16.7 | 13 ; 20.3 | 50 | 0 | - | 170 | 6.2 | 2.7 ; 9.7 | 152 | 31.3 | 24.3 ; 38.4 |
| July | 337 | 19.2 | 15.2 ; 23.2 | 40 | 2.5 | 0 ; 5.2 | 130 | 4.2 | 1.3 ; 7.1 | 167 | 31.1 | 24.5 ; 37.6 |
| August | 463 | 17.5 | 14.3 ; 20.8 | 79 | 0 | - | 176 | 3.4 | 1.1 ; 5.8 | 208 | 32.5 | 26.4 ; 38.5 |
| September | 460 | 20.9 | 17.6 ; 24.1 | 72 | 0 | - | 199 | 3.2 | 1.1 ; 5.3 | 189 | 47.2 | 40.5 ; 53.9 |
| October | 141 | 18.3 | 12.9 ; 23.8 | 22 | 0 | - | 56 | 11 | 3.5 ; 18.4 | 63 | 37.1 | 28.1 ; 46.2 |
| November | 202 | 23.1 | 17.8 ; 28.4 | 38 | 0 | - | 67 | 6.4 | 1 ; 11.9 | 97 | 48.7 | 39.4 ; 58 |
| December | 183 | 17.1 | 12 ; 22.2 | 48 | 1.6 | 0 ; 3.3 | 70 | 4.4 | 0.3 ; 8.6 | 65 | 45.1 | 33 ; 57.2 |

## *Salmonella* Typhimurium

MSs with holdings where *Salmonella* Typhimurium isolated = Austria, Belgium, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Italy, Poland, Portugal, Spain, the Netherlands, the United Kingdom

| Factor | Category level | EU | | | MS with isolates | | |
|---|---|---|---|---|---|---|---|
| | | N obs | % pos | 95% CI | N obs | % pos | 95% CI |
| **Overall** | | 26,656 | 0.8 | 0.6 ; 0.9 | 24,766 | 0.8 | 0.6 ; 1.0 |
| **Sample-level** | | | | | | | |
| Sample type | faeces | 19,040 | 0.7 | 0.5 ; 0.9 | 17,690 | 0.7 | 0.5 ; 0.9 |
| | Dust | 7,616 | 1.0 | 0.7 ; 1.1 | 7,076 | 1.0 | 0.7 ; 1.2 |
| **Overall** | | 3,808 | 2.5 | 2.1 ; 3.0 | 3,538 | 2.6 | 2.1 ; 3.1 |
| **Flock-level** | | | | | | | |
| Flock production type | cage | 2,303 | 3.4 | 2.7 ; 4.1 | 2,143 | 3.5 | 2.8 ; 4.2 |
| | barn | 600 | 1.9 | 0.8 ; 3.1 | 586 | 2.0 | 0.8 ; 3.1 |
| | free-range standard | 633 | 1.1 | 0.3 ; 2.0 | 546 | 1.2 | 0.3 ; 2.1 |
| | organic | 272 | 0.4 | 0.0 ; 0.8 | 263 | 0.4 | 0.0 ; 0.8 |
| Age type flock | Homogeneous age | 3,670 | 2.6 | 2.1 ; 3.1 | 3,426 | 2.7 | 2.2 ; 3.2 |
| | Mixed age | 138 | 0.9 | 0.0 ; 1.3 | 112 | 1.0 | 0.0 ; 2.0 |
| Age of hens at sampling (weeks) | Q1 : 26, 61 [a] | 970 | 2.4 | 1.5 ; 3.3 | 919 | 2.4 | 1.5 ; 3.4 |
| | Q2 : 62, 68 | 997 | 2.2 | 1.3 ; 3.0 | 914 | 2.2 | 1.3 ; 3.1 |
| | Q3 : 69, 75 | 944 | 2.6 | 1.5 ; 3.7 | 853 | 2.7 | 1.6 ; 3.8 |
| | >Q3 : 76, 150 | 897 | 3.0 | 2.1 ; 3.9 | 852 | 3.1 | 2.1 ; 4.0 |
| Vaccination status | Unvaccinated | 2,456 | 3.0 | 2.4 ; 3.6 | 2,203 | 3.1 | 2.5 ; 3.8 |
| | Vacc with STM vaccine | 373 | 1.8 | 0.5 ; 3.1 | 372 | 1.8 | 0.5 ; 3.2 |
| | Vacc with non-STM vaccine | 979 | 1.9 | 1.1 ; 2.8 | 963 | 1.9 | 1.1 ; 2.8 |
| Medication status | No antimicrobials used during the last two weeks | 3,768 | 2.5 | 2.1 ; 3.0 | 3,498 | 2.6 | 2.1 ; 3.1 |
| | Antimicrobials used during the last two weeks | 40 | 2.5 | 0.0 ; 5.1 | 40 | 2.5 | 0.0 ; 5.1 |
| **Holding-level** | | | | | | | |
| Number of hens in holding | Q1 : 61; 3,000 | 997 | 1.6 | 0.9 ; 2.3 | 891 | 1.7 | 0.9 ; 2.4 |
| | Q2 : >3,000; 8,000 | 938 | 2.5 | 1.6 ; 3.5 | 885 | 2.5 | 1.6 ; 3.5 |
| | Q3 : >8,000; 23,000 | 925 | 2.1 | 1.3 ; 3 | 879 | 2.3 | 1.4 ; 3.2 |
| | >Q3 : >23,000; 1,200,000 | 948 | 3.9 | 2.7 ; 5.1 | 883 | 4.0 | 2.7 ; 5.2 |
| **General** | | | | | | | |
| Days to bacteriological analysis | 0-1 | 2,131 | 2.2 | 1.6 ; 2.8 | 1,961 | 2.3 | 1.6 ; 3.0 |
| | 2 | 545 | 2.3 | 1.2 ; 3.4 | 468 | 2.4 | 1.2 ; 3.6 |
| | 3 | 517 | 4.4 | 2.9 ; 5.8 | 506 | 4.4 | 2.9 ; 5.9 |
| | 4-7 | 615 | 2.7 | 1.5 ; 3.9 | 603 | 2.7 | 1.5 ; 4.0 |
| Months of sampling | January | 267 | 1.8 | 0.4 ; 3.1 | 252 | 1.8 | 0.4 ; 3.2 |
| | February | 259 | 0.6 | 0.1 ; 1.2 | 246 | 0.6 | 0.1 ; 1.2 |
| | March | 355 | 5.4 | 2.9 ; 8.0 | 326 | 5.6 | 3.0 ; 8.3 |
| | April | 407 | 2.4 | 1.0 ; 3.7 | 366 | 2.5 | 1.0 ; 3.9 |
| | May | 362 | 1.7 | 0.4 ; 3.0 | 335 | 1.8 | 0.4 ; 3.1 |
| | June | 372 | 3.8 | 2.1 ; 5.5 | 347 | 4.0 | 2.1 ; 5.7 |
| | July | 337 | 3.3 | 1.5 ; 5.2 | 304 | 3.5 | 1.6 ; 5.4 |
| | August | 463 | 0.9 | 0.2 ; 1.6 | 409 | 1.0 | 0.2 ; 1.7 |
| | September | 460 | 2.1 | 0.9 ; 3.3 | 440 | 2.2 | 0.9 ; 3.4 |
| | October | 141 | 3.0 | 1.2 ; 4.9 | 139 | 3.0 | 1.2 ; 4.9 |
| | November | 202 | 3.7 | 1.2 ; 6.2 | 198 | 3.8 | 1.2 ; 6.3 |
| | December | 183 | 1.7 | 0.0 ; 3.4 | 176 | 1.7 | 0.0 ; 3.4 |

Q1, Q2, and Q3: Quartiles 1 to 3

[a] Minimum, maximum category value

## Serovars other than *Salmonella* Enteritidis and Typhimurium

MSs with holdings where serovars other than *Salmonella* Enteritidis-Typhimurium were isolated (Austria, Belgium, Cyprus, the Czech Republic, Denmark, Estonia, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Poland, Portugal, Slovenia, Spain, the Netherlands and the United Kingdom)

| Factor | Category level | EU | | | MS with isolates | | |
|---|---|---|---|---|---|---|---|
| | | N obs | % pos | 95% CI | N obs | % pos | 95% CI |
| **Overall** | | 26,656 | 7.6 | 7.1 ; 8.2 | 24,864 | 8.0 | 7.4 ; 8.5 |
| **Sample-level** | | | | | | | |
| Sample type | faeces | 19,040 | 6.8 | 6.2 ; 7.3 | 17,760 | 7.0 | 6.5 ; 7.6 |
| | Dust | 7,616 | 9.9 | 9.1 ; 10.6 | 7,104 | 10.3 | 9.5 ; 11.0 |
| **Overall** | | 3,808 | 18.3 | 17.2 ; 19.4 | 3,552 | 19.0 | 17.8 ; 20.2 |
| **Flock-level** | | | | | | | |
| Flock production type | cage | 2,303 | 27.7 | 26.0 ; 29.4 | 2,093 | 29.4 | 27.6 ; 31.2 |
| | barn | 600 | 7.2 | 5.1 ; 9.2 | 574 | 7.3 | 5.3 ; 9.4 |
| | free-range standard | 633 | 3.6 | 2.2 ; 5.0 | 629 | 3.6 | 2.2 ; 5.0 |
| | organic | 272 | 6.9 | 3.4 ; 10.3 | 256 | 7.1 | 3.5 ; 10.7 |
| Age type flock | Homogeneous age | 3,670 | 18.4 | 17.2 ; 19.5 | 3,419 | 19.1 | 18.0 ; 20.3 |
| | Mixed age | 138 | 15.6 | 10.1 ; 21.1 | 133 | 16.0 | 10.3 ; 21.5 |
| Age of hens at sampling (weeks) | Q1 : 26, 61 [a] | 970 | 15.4 | 13.3 ; 17.5 | 894 | 16.2 | 14.0 ; 18.4 |
| | Q2 : 62, 68 | 997 | 12.4 | 10.5 ; 14.4 | 915 | 13.1 | 11.0 ; 15.1 |
| | Q3 : 69, 75 | 944 | 15.0 | 12.7 ; 17.2 | 863 | 15.8 | 13.5 ; 18.2 |
| | >Q3 : 76, 150 | 897 | 30.9 | 28.1 ; 33.4 | 880 | 31.2 | 28.5 ; 34.0 |
| Vaccination status | Unvaccinated | 2,190 | 22.1 | 20.6 ; 23.7 | 1,934 | 24.1 | 22.4 ; 25.8 |
| | Vacc with any *Salmonella* vaccine | 1,618 | 14.5 | 12.9 ; 16.1 | 1,618 | 14.5 | 12.9 ; 16.1 |
| Medication status | No antimicrobials used during the last two weeks | 3,768 | 18.3 | 17.2 ; 19.9 | 3,512 | 19.1 | 17.9 ; 20.2 |
| | Antimicrobials used during the last two weeks | 40 | 15.9 | 5.3 ; 26.6 | 40 | 15.9 | 5.3 ; 26.6 |
| **Holding-level** | | | | | | | |
| Number of hens in holding | Q1 : 61; 3,000 | 997 | 7.2 | 5.7 ; 8.8 | 894 | 7.7 | 6.1 ; 9.4 |
| | Q2 : >3,000; 8,000 | 938 | 11.2 | 9.3 ; 13.2 | 882 | 12.1 | 10.1 ; 14.2 |
| | Q3 : >8,000; 23,000 | 925 | 20.0 | 17.6 ; 22.4 | 898 | 21.3 | 18.8 ; 23.7 |
| | >Q3 : >23,000; 1,200,000 | 948 | 35.0 | 32.2 ; 37.8 | 878 | 35.9 | 33.0 ; 38.8 |
| **General** | | | | | | | |
| Days to bacteriological analysis | 0-1 | 2,131 | 15.9 | 14.4 ; 17.4 | 1,917 | 16.9 | 15.3 ; 18.5 |
| | 2 | 545 | 17.8 | 14.5 ; 21.1 | 515 | 18.4 | 15.0 ; 21.8 |
| | 3 | 517 | 32.0 | 28.2 ; 35.3 | 514 | 32.2 | 28.9 ; 35.5 |
| | 4-7 | 615 | 17.7 | 15.1 ; 20.4 | 606 | 17.9 | 15.2 ; 20.5 |
| Months of sampling | January | 267 | 12.1 | 8.7 ; 15.6 | 247 | 12.7 | 9.1 ; 16.3 |
| | February | 259 | 22.6 | 17.8 ; 27.5 | 238 | 23.8 | 18.8 ; 29.0 |
| | March | 355 | 21.2 | 17.1 ; 25.3 | 335 | 21.9 | 17.7 ; 26.1 |
| | April | 407 | 16.1 | 12.8 ; 19.4 | 400 | 16.3 | 12.9 ; 19.6 |
| | May | 362 | 19.1 | 15.2 ; 23.0 | 345 | 19.6 | 15.6 ; 23.6 |
| | June | 372 | 17.1 | 13.4 ; 20.8 | 360 | 17.4 | 13.7 ; 21.2 |
| | July | 337 | 12.6 | 9.4 ; 15.9 | 329 | 12.8 | 9.5 ; 16.1 |
| | August | 463 | 15.9 | 12.8 ; 19.0 | 436 | 16.4 | 13.2 ; 19.7 |
| | September | 460 | 22.7 | 19.0 ; 26.3 | 412 | 24.2 | 20.3 ; 28.1 |
| | October | 141 | 25.3 | 19.4 ; 31.3 | 129 | 26.9 | 20.6 ; 33.2 |
| | November | 202 | 26.2 | 20.8 ; 31.7 | 170 | 29.4 | 23.3 ; 35.5 |
| | December | 183 | 14.3 | 9.3 ; 19.2 | 151 | 16.1 | 10.5 ; 21.7 |

Q1, Q2, and Q3: Quartiles 1 to 3

[a] Minimum, maximum category value

**Annex X. Most frequently identified *Salmonella* Enteritidis phage types in MSs, in the EU laying hens baseline study, 2004-2005**

| **Austria**[1] | ***S.* Enteritidis (N=157)** | | | **No of holdings where** |
|---|---|---|---|---|
| | **phage type** | **N** | **%** | **the phage type was detected** |
| | PT 4 | 48 | 30.6 | 16 |
| | PT 8 | 43 | 27.4 | 10 |
| | PT 7 | 30 | 19.1 | 8 |
| | PT 21 | 18 | 11.5 | 5 |
| | PT 23 | 5 | 3.2 | 3 |
| | PT 1 | 4 | 2.5 | 2 |
| | PT 19 | 5 | 3.2 | 1 |
| | PT 6 | 1 | 0.6 | 1 |
| | RDNC | 3 | 1.9 | 2 |

[1]. one *S.* Enteritidis isolate not phagetyped

| **Czech Republic**[1] | ***S.* Enteritidis (N=38)** | | | **No of holdings where** |
|---|---|---|---|---|
| | **phage type** | **N** | **%** | **the phage type was detected** |
| | PT 8 | 17 | 44.7 | 17 |
| | PT 23 | 8 | 21.1 | 8 |
| | PT 6a | 3 | 7.9 | 3 |
| | PT 13a | 2 | 5.3 | 2 |
| | PT 7 | 2 | 5.3 | 2 |
| | PT 1 | 1 | 2.6 | 1 |
| | PT 12 | 1 | 2.6 | 1 |
| | PT 13 | 1 | 2.6 | 1 |
| | PT 21b | 1 | 2.6 | 1 |
| | PT 6 | 1 | 2.6 | 1 |
| | non typeable | 1 | 2.6 | 1 |

[1]. 94 *S.* Enteritidis isolates not phagetyped

| Denmark[1] | *S.* Enteritidis (N=5) | | | No of holdings where |
|---|---|---|---|---|
| | phage type | N | % | the phage type was detected |
| | PT 8 | 2 | 40.0 | 1 |
| | PT 28 | 1 | 20.0 | 1 |
| | PT 29 | 1 | 20.0 | 1 |
| | non typeable | 1 | 20.0 | 1 |

[1]. one *S.* Enteritidis isolate not phagetyped

| Germany | *S.* Enteritidis (N=357) | | | No of holdings where |
|---|---|---|---|---|
| | phage type | N | % | the phage type was detected |
| | PT 4 | 246 | 68.9 | 96 |
| | PT 8 | 49 | 13.7 | 16 |
| | PT 1 | 13 | 3.6 | 4 |
| | PT 21 | 5 | 1.4 | 2 |
| | PT 6 | 5 | 1.4 | 2 |
| | PT 35 | 4 | 1.1 | 2 |
| | PT 6a | 3 | 0.8 | 2 |
| | PT 30 | 2 | 0.6 | 2 |
| | PT 7 | 2 | 0.6 | 2 |
| | PT 5a | 3 | 0.8 | 1 |
| | PT 19 | 2 | 0.6 | 1 |
| | PT 21c | 2 | 0.6 | 1 |
| | PT 25 | 2 | 0.6 | 1 |
| | PT 14b | 1 | 0.3 | 1 |
| | PT 2 | 1 | 0.3 | 1 |
| | PT 23 | 1 | 0.3 | 1 |
| | PT 4a | 1 | 0.3 | 1 |
| | PT 4b | 1 | 0.3 | 1 |
| | PT 7a | 1 | 0.3 | 1 |
| | RDNC | 10 | 2.8 | 6 |
| | non typeable | 3 | 0.8 | 2 |

| Italy[1] | *S.* Enteritidis (N=20) | | | No of holdings where |
|---|---|---|---|---|
| | phage typ | N | % | the phage type was detected |
| | PT 14b | 11 | 55.0 | 4 |
| | PT 4 | 3 | 15.0 | 2 |
| | PT 7a | 6 | 30.0 | 1 |

[1]. 21 *S.* Enteritidis isolates not phagetyped

| Lithuania[1] | *S.* Enteritidis (N=1) | | | No of holdings where |
|---|---|---|---|---|
| | phage type | N | % | the phage type was detected |
| | non typeable | 1 | 100.0 | 1 |

[1]. seven *S.* Enteritidis isolates not phagetyped

| The Netherlands | *S.* Enteritidis (N=98) | | | No of holdings where |
|---|---|---|---|---|
| | phage type | N | % | the phage type was detected |
| | PT 4 | 16 | 16.3 | 11 |
| | PT 7 | 8 | 8.2 | 4 |
| | PT 6 | 5 | 5.1 | 4 |
| | PT 8 | 5 | 5.1 | 3 |
| | PT 21 | 8 | 8.2 | 2 |
| | PT 1 | 5 | 5.1 | 2 |
| | PT 25 | 5 | 5.1 | 1 |
| | PT 29 | 1 | 1.0 | 1 |
| | non typeable | 45 | 45.9 | 19 |

| The United Kingdom | *S.* Enteritidis (N=114) | | | No of holdings where |
|---|---|---|---|---|
| | phage type | N | % | the phage type was detected |
| | PT 4 | 41 | 36.0 | 15 |
| | PT 35 | 22 | 19.3 | 8 |
| | PT 6 | 16 | 14.0 | 7 |
| | PT 7 | 9 | 7.9 | 5 |
| | PT 5a | 2 | 1.8 | 2 |
| | PT 1 | 2 | 1.8 | 1 |
| | PT 12 | 6 | 5.3 | 1 |
| | PT 14b | 1 | 0.9 | 1 |
| | PT 24 | 1 | 0.9 | 1 |
| | PT 5c | 1 | 0.9 | 1 |
| | PT 6a | 4 | 3.5 | 1 |
| | non typeable | 9 | 7.9 | 7 |

**Annex XI. Most frequently identified *Salmonella* Typhimurium phage types in MSs, in the EU laying hens baseline study, 2004-2005**

| Austria | *S.* Typhimurium (N=12) | | | No of holdings where |
|---|---|---|---|---|
| | phage type | N | % | the phage type was detected |
| | DT 104 | 7 | 58.3 | 1 |
| | DT 193 | 3 | 25.0 | 1 |
| | RDNC | 2 | 16.7 | 2 |

| Czech Republic[1] | *S.* Typhimurium (N=3) | | | No of holdings where |
|---|---|---|---|---|
| | phage type | N | % | the phage type was detected |
| | RDNC | 1 | 33.3 | 1 |
| | non typeable | 2 | 66.7 | 2 |

[1]. five *S.* Typhimurium isolates not phagetyped

| Finland | *S.* Typhimurium (N=1) | | | No of holdings where |
|---|---|---|---|---|
| | phage type | N | % | the phage type was detected |
| | DT 1 | 1 | 100.0 | 1 |

| Germany | *S.* Typhimurium (N=29) | | | No of holdings where |
|---|---|---|---|---|
| | phage type | N | % | the phage type was detected |
| | DT 104 | 17 | 73.9 | 5 |
| | DT 120 | 3 | 13.0 | 2 |
| | DT 195 | 1 | 4.3 | 1 |
| | DT 7 | 1 | 4.3 | 1 |
| | DT 9 | 1 | 4.3 | 1 |
| | RDNC | 6 | 26.1 | 4 |

| Italy[1] | *S.* Typhimurium (N=5) | | | No of holdings where |
|---|---|---|---|---|
| | phage type | N | % | the phage type was detected |
| | DT 1 | 3 | 60.0 | 3 |
| | DT 193 | 1 | 20.0 | 1 |
| | DT 99 | 1 | 20.0 | 1 |

[1]. 22 *S.* Typhimurium isolates not phagetyped

| The Netherlands[1] | *S.* Typhimurium (N=6) | | | No of holdings where |
|---|---|---|---|---|
| | phage type | N | % | the phage type was detected |
| | DT 104 | 6 | 100.0 | 4 |

[1]. seven *S.* Typhimurium isolates not phagetyped

| The United Kingdom | *S.* Typhimurium (N=22) | | | No of holdings where |
|---|---|---|---|---|
| | phage type | N | % | the phage type was detected |
| | DT 104 | 13 | 59.1 | 4 |
| | DT 49 | 6 | 27.3 | 1 |
| | DT 1 | 1 | 4.5 | 1 |
| | DT 2a | 1 | 4.5 | 1 |
| | DT 56 | 1 | 4.5 | 1 |