# EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on a quantitative estimation of the public health impact of setting a new target for the reduction of Salmonella in broilers

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SCIENTIFIC OPINION

# Scientific Opinion on a quantitative estimation of the public health impact of setting a new target for the reduction of *Salmonella* in broilers [1]

**EFSA Panel on Biological Hazards (BIOHAZ)**[2, 3]

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## ABSTRACT

This assessment relates the percentage of broiler-associated human salmonellosis cases to different *Salmonella* prevalences in broiler flocks in the European Union. It considers the contribution and relevance of different *Salmonella* serovars found in broilers to human salmonellosis. The model developed to provide quantitative estimates, which is based on the microbial-subtyping approach, considers 22 Member States, four animal-food sources of *Salmonella* (broilers, laying hens, pigs and turkeys) and 23 *Salmonella* serovars. The model (called the 'Broiler Target Salmonella Attribution Model' or BT-SAM model) employes data from the EU Baseline Surveys and EU statutory monitoring on *Salmonella* in animal-food sources, data on incidence of human salmonellosis and food availability data. It is estimated that around 2.4%, 65%, 28% and 4.5% of the human salmonellosis cases are attributable to broilers, laying hens (eggs), pigs and turkeys respectively. Of the broiler-associated human salmonellosis cases, around 42% and 23% are estimated to be due to the serovars *Salmonella* Enteritidis and *Salmonella* Infantis respectively, while other serovars individually contributed less than 5%. Different scenarios are presented showing changes in the percentage of broiler-associated human salmonellosis cases under different prevalences of *Salmonella* in broiler flocks. Compared to 2006, the 2009 *Salmonella* in broiler flocks prevalence has achieved a reduction of 69% in the number of broiler-associated human salmonellosis cases. When comparing the results of the adjusted prevalences for *Salmonella* Enteritidis and *Salmonella* Typhimurium as reported in 2009 with a theoretical combined prevalence of 1% for these two serovars, the difference between the percentages of broiler-associated cases is small. However, when adjusting the combined prevalence of all serovars to 1%, a large reduction in the percentage of broiler-associated cases compared to the one achieved with the two previous serovars only is expected. Uncertainty and data limitations are discussed, including recommendations on how these could be overcome.



## KEY WORDS

*Salmonella*, poultry, broiler, source attribution, microbial subtyping, targets.

[1] On request from the European Commission. Question No EFSA-Q-2008-293, adopted on 7 July 2011.

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## SUMMARY

Following a request from the European Commission, the Scientific Panel on Biological Hazards (BIOHAZ) was asked to deliver a Scientific Opinion on a quantitative estimation of the public health impact of setting a new target for the reduction of *Salmonella* in broilers. Specifically, EFSA was asked to assess the relative public health impact if a new target for reduction of *Salmonella* is set in broilers being 1% or less remaining positive for all *Salmonella* serovars with public health significance, compared to (1) the theoretical prevalence at the end of the transitional period (1% or less flocks remaining positive for *Salmonella* Enteritidis and/or *Salmonella* Typhimurium), and (2) the real prevalence in 2009 reported by the Member States (MSs).

For this task, the BIOHAZ Panel was supported by the work of a contractor that developed a source attribution model providing estimates for the quantitative contribution of broilers and other major animal-food sources to the estimated true burden of human salmonellosis in the EU. The model was based on the so-called microbial subtyping approach, which allows for distinguishing between the different serovars. The basic principle is to compare the serovar distributions observed in different animal-food sources with the serovar distribution found in humans. The full Technical Report submitted to EFSA by the contractor provides detailed information on the modeling approach and results.

The model considered the following data: (i) the EU-wide *Salmonella* Baseline Surveys on broiler flocks, broiler carcasses, turkey flocks and slaughter pigs, (ii) the results from the harmonised EU monitoring in broiler and laying hen flocks in 2009, (iii) the reported cases of human salmonellosis in EU in 2007 to 2009 by MSs as provided by the European Centre for Disease Prevention and Control[4] (ECDC), and (iv) the amount of each food source available for consumption by MS as estimated from EUROSTAT data on production, import and export. The model included data from 22 MSs, four animal-food *Salmonella* sources (broilers, laying hens, pigs and turkeys) and 23 individual serovars. To take account for differences in underreporting of human salmonellosis cases between MSs, MS-specific underreporting factors were calculated and applied in the model. Some sources of *Salmonella* (e.g. cattle/beef) were not included in the model due to lack of data. The possible influence of this is discussed.

Initially, a model applying prevalence data from the Baseline Survey conducted in broiler flocks in 2005-2006 was developed. This model is referred to as the 'Broiler Target Salmonella Attribution Model' or BT-SAM model throughout the Opinion. In order to answer the Terms of Reference, different scenarios where *Salmonella* prevalences in broiler flocks were changed were developed and the results compared to the results of the BT-SAM model.

The BIOHAZ Panel concluded that based on the results of the BT-SAM model 2.4% (95% CI: 1.8-3.4) of all human salmonellosis cases (i.e. estimated true number of cases when accounting for underreporting) in the EU were attributed to broilers. Around half of the broiler-associated human salmonellosis cases were caused by serovars other than the currently regulated serovars. *Salmonella* Enteritidis and *Salmonella* Infantis constituted 42% and 23% of all broiler-associated cases respectively. *Salmonella* Hadar, *Salmonella* Typhimurium, *Salmonella* Kentucky and *Salmonella* Virchow constituted individually between 4% and 5% of all broiler-associated cases. Other serovars constituted less than 4% on an individual basis.

For the other *Salmonella* sources included, the model estimated that around 65% (95% CI: 63-67), 28% (95% CI: 27-30) and 4.5% (95% CI: 4-5) of the estimated number of human salmonellosis cases could be attributed to laying hens (eggs), pigs and turkeys, respectively. The results of the model indicate that the majority of the *Salmonella* Enteritidis infections are related to the laying hen reservoir (i.e. consumption of eggs), whereas *Salmonella* Typhimurium infections originate primarily from the pig reservoir.


[4] ECDC, TESSy Release 1 (06/07/2010) and 2 (28/10/2010 and updated on 05/05/2011). ECDC has no responsibility for the results and conclusions when disseminating the results of the work employing TESSy data supplied by ECDC.

The Panel concluded that the situation in 2009 already showed a considerable improvement compared to the results of the BT-SAM model with a reduction of 69% in the number of broiler-associated human salmonellosis cases compared to the situation in 2006. The Panel further concluded that: (1) Considering that the prevalence of *Salmonella* Enteritidis and *Salmonella* Typhimurium is as reported by the Member States in 2009 (but keeping the prevalence for the other 21 serovars as per the 2005-2006 Baseline Survey in broiler flocks), an estimated reduction in the number of broiler-associated human salmonellosis cases of 26% compared to the situation in 2006 is expected; (2) considering that the current target of the EU control programme of *Salmonella* in broiler flocks would be met (i.e. the combined prevalence of *Salmonella* Enteritidis and *Salmonella* Typhimurium being 1% or less), and keeping the prevalence for the other 21 serovars as per the 2005-2006 Baseline Survey in broiler flocks, an estimated reduction in the number of broiler-associated human salmonellosis cases of 25% compared to the situation in 2006 is expected; (3) Considering that an EU-wide target of maximum of 1% of flocks remaining positive for the all the *Salmonella* serovars considered in the model would be met, an estimated reduction in the number of broiler-associated human salmonellosis cases of 93% compared to the situation in 2006 is expected.

The Panel emphasised that the EU statutory monitoring in the Member States is likely to have a lower sensitivity in detecting positive flocks than the conducted EU-wide Baseline Surveys. For this reason, the estimated reductions in number of human salmonellosis cases are overestimated at the EU-level. Furthermore, the individual MS contributions to the estimated reductions vary greatly.

The BIOHAZ Panel finally concluded that the main factors contributing to the uncertainty of the model results beyond the statistical uncertainty are the lack of harmonised monitoring of human salmonellosis in the EU as well as the different levels of serovar detail reported in both the human and animal food source data. These uncertainties could not be statistically quantified with the model employed to support this Scientific Opinion.

The Panel makes a series of recommendations related to the establishment of active surveillance of human salmonellosis in all MSs, including efforts to quantify the level of under-ascertainment and underreporting. For both human and animal-food surveillance data it is recommended to make available more comparable *Salmonella* subtyping data for more accurate future modeling and trend analyses. In addition, it is recommended to repeat the subtyping modelling approach on a regular basis (i.e. every 3 to 5 years) in order to follow the progress of *Salmonella* control and the trends in the sources of human salmonellosis.

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## BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The presence of *Salmonella* in poultry populations is considered as a risk factor for the presence of *Salmonella* in meat and eggs. Targets are being set for the reduction of certain *Salmonella* serovars in different poultry populations within the frame of Regulation (EC) No 2160/2003[5] on the control of zoonoses. As a transitional measure, a limited number of *serovar*s have been considered for reduction during the first three years of the control programme. Before the end of this period, a review of the *serovar*s should be considered.

As regards broilers, Regulation (EC) No 646/2007[6] transitionally sets a target for reduction being less than 1% or less flocks remaining positive for *Salmonella* Enteritidis or *Salmonella* Typhimurium by the end of 2011. The Regulation also harmonises the monitoring in broilers in all Member States from the beginning of 2009 on. Therefore, comparable prevalence data of all Member States are available. These prevalence data will be forwarded by Member States to EFSA's Zoonoses Data Collection unit.

For the setting of a new target for reduction of *Salmonella* beyond 2011, a cost/benefit analysis should be carried out (See flow chart in next page). Such benefit should be defined as a beneficial public health impact of a possible new target.

## TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The EFSA is asked to assess the relative public health impact if a new target for reduction of *Salmonella* is set in broilers being 1% or less remaining positive for all *Salmonella* serovars with public health significance, compared to:

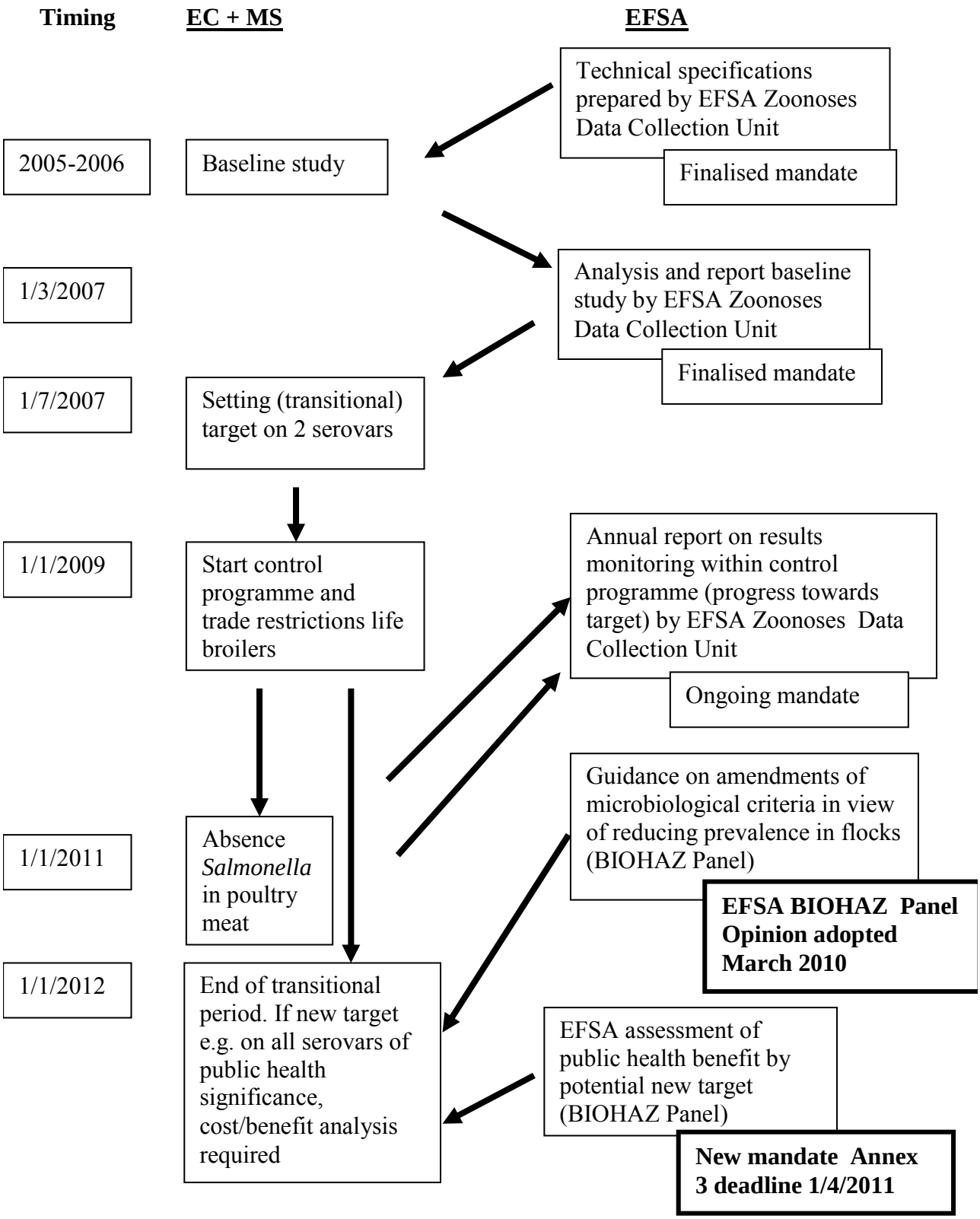
- the theoretical prevalence at the end of the transitional period (1% or less flocks remaining positive for *Salmonella* Enteritidis or *Salmonella* Typhimurium), and
- the real prevalence in 2009 to be reported by the Member States.

The *Salmonella* serovars with public health significance should be determined by the EFSA taking into account the criteria laid down in Annex III to Regulation (EC) No 2160/2003.

[5] OJ L 325, 12.12.2003, p. 1. Regulation as last amended by Commission Regulation (EC) No 1237/2007 (OJ L 208, 24.10.2007, p. 5)

[6] OJ L 151, 13.6.2007, p. 21.

## Flowchart *Salmonella* control programmes broilers and needs for EFSA input



## ASSESSMENT

### 1. Introduction

This EFSA Scientific Opinion is the third of a series of quantitative estimates of the impact of setting new targets for the reduction of *Salmonella* in certain poultry populations (*Gallus gallus*, and from now on 'broiler' will refer to the species of concern in this Opinion), which were requested by the European Commission in a mandate back in April 2008.

The first of the Opinions, which was published in April 2009, dealt with a request to provide a quantitative estimation of the impact of setting a new target for the reduction of the prevalence of *Salmonella* in breeding hens of *Gallus gallus* (EFSA, 2009). A second Opinion, published in April 2010 provided an estimate of the public health impact of setting a new target for the reduction of *Salmonella* in laying hens (EFSA, 2010d).

In the current Opinion, a quantitative estimate of the public health impact of setting a new target for the reduction of *Salmonella* in broilers is considered. According to the Terms of Reference (ToR) presented in the background as provided by the European Commission, EFSA is asked to provide an estimate of the public health impact of different flock prevalence values in broilers (i.e. theoretical *vs.* reported for the year 2009) of different *Salmonella* serovar groups (i.e. *S.* Enteritidis and *S.* Typhimurium vs. all *Salmonella* serovars with 'public health significance').

The determination of the serovars with public health significance has been done following the criteria laid down in Annex III to Regulation (EC) No 2160/2003, as requested in the ToR. It follows a similar methodological approach as in the previous related EFSA Opinions (EFSA, 2009, 2010d), but in this occasion considering the particular context of broiler meat production.

The quantitative aspects considered in this Opinion have been supported by the work of a contractor selected by means of an EFSA open call for tender (CFT/BIOHAZ/2010/02, call published on 5 December 2009). The report of the work carried out by this contractor is published as a separate document (VOSE, 2011). There were continuous consultation and follow up between the Contractor and the members of the *ad hoc* working group (WG) who elaborated the draft Scientific Opinion. This consultation mainly aimed at (i) achieving and understanding by the WG of the modelling approach, (ii) providing the Contractor with an evaluation by the WG experts of the assumptions made in the model and (iii) understanding and agreeing on data sources to be used for providing the quantitative estimates. Cross-reference is made in this Opinion to the contractor's report when it supports the statements made. Moreover, the contractor's report should be read as part of this Scientific Opinion.

### 2. *Salmonella* in humans in the EU

A total of 108,614 confirmed cases of human salmonellosis were reported from 27 EU Member States (MSs) through the European Surveillance System (TESSy) in 2009 (EFSA and ECDC, 2011). The EU notification rate was 23.7 cases per 100,000 population, ranging from 2.1 in Portugal to 100.1 confirmed cases per 100,000 population in Czech Republic. As in previous years, *S.* Enteritidis and *S.* Typhimurium were the most frequently reported serovars (75.6% of all notified serovars).

The overall reported incidence of human salmonellosis has been decreasing from 2006 to 2009, which is mainly explained by a downward trend in the number of *S.* Enteritidis infections (see section 4.1. and Table 3). However, comparison of the notification rates between countries should be made with caution because of the different degrees of underreporting that exist between MSs (EFSA, 2010d). The importance of underreporting is discussed in more detail below. Comparison between years within a MS is, in general, more valid.

The total number of reported cases includes sporadic, travel and outbreak-related infections. However, when assessing the public health importance of a given food source (e.g. broiler meat), and especially when assessing the expected reduction in human cases due to a certain control strategy, it is important

to consider the proportion of cases that are associated with travel abroad and/or larger outbreaks. The effect of control efforts implemented at the EU level should be reflected in a decline in the total number of salmonellosis cases, including those acquired during intra-EU travelling. Cases acquired outside the EU can not be expected to be reduced as a consequence of EU efforts to control *Salmonella* in primary production or processing. Large and possible international outbreaks may influence the total incidence in the EU as in individual MSs, but as they are often caused by failures in the production of a single or more (batches of) food product originating from the same producer, the EU and national control efforts will probably only affect such events to a minor degree if at all. In other words, EU control efforts are in general expected only to have a direct effect on the number of EU-acquired and sporadic cases.

Overall, in 2009, the reported proportion of reported cases acquired abroad was around 10.5%, whereas the proportion of domestically acquired cases was around 62.4%. However, these proportions varied greatly among MSs and for some countries such as Sweden and Finland, the travel-related cases represented the majority of all salmonellosis cases. The proportion of cases with an unknown location of origin represented around 27.1% of confirmed cases, but in nine MSs the proportion of unknown location of origin is reported to be 100% (EFSA and ECDC, 2011). Data on domestic versus travel-related cases are, therefore, often incomplete, but should, as far as possible, be accounted for in the analysis of the public health impact of a given source. In addition, the continuous reporting of these data may indicate common cultural features in some geographical areas and also indicate something about the effect of national control measures.

A total of 324 verified *Salmonella* outbreaks involving 4,500 confirmed cases were reported by MSs in 2009 (EFSA and ECDC, 2011). The verified outbreaks were reported primarily by France, Poland and Spain. In total, 22% of human cases in verified outbreaks reported by MSs were hospitalised and the human case fatality rate was 0.13%. *S*. Enteritidis was the predominant serovar associated with the *Salmonella* outbreaks, which is similar to previous years (EFSA and ECDC, 2009, 2010). In 2009, *S*. Enteritidis accounted for 59.6% of all verified *Salmonella* outbreaks, 58.2% of all human *Salmonella* cases, 73.1% of all hospitalisations and 66.7% of all case fatalities. *S*. Typhimurium was associated with 15.7% of the verified outbreaks, 20.2% of all human cases, 10.9% of all hospitalisations and 33.3% of all deaths in 2009. In 17.9% of the verified outbreaks caused by *Salmonella*, the serovar was not reported or was unknown. Only 14.3% of the outbreaks of *S*. Enteritidis or *S*. Typhimurium included information of the isolated phage type (EFSA and ECDC, 2011).

The reporting of outbreaks by the different MSs depends very much on the resources in place for handling these incidents. Furthermore, large (or spectacular) outbreaks will have a greater probability of being detected by the surveillance systems in a MS than smaller outbreaks. The chance for verifying the causative agent is also inherently associated with the incriminated food vehicle and food source. If the combination of food vehicle and the causative agent is frequently linked and associated with outbreaks (e.g. *S*. Enteritidis in eggs) it may be anticipated that the chance for verifying the outbreak will be greater.

The previous EFSA Scientific Opinion dealing with a similar question, but related to laying hens, provided detailed information on underreporting of human salmonellosis (EFSA, 2010d). Details of the reporting system for human salmonellosis in the EU and the results up to 2007 can also be found in the previous EFSA Opinion relating to targets for breeding hens (EFSA, 2009) and in the Community Summary Report (EFSA, 2010c). These documents also describe issues related to underreporting of human salmonellosis and indicate that the true burden may be considerably larger than the reported incidence, albeit that the level of underreporting varies strongly between MSs (de Jong and Ekdahl, 2006). “Multipliers” (i.e. the ratio between true and reported cases) estimated in the 1990's range from 3.2 for the UK, through 13.2 for the Netherlands to 38.6 for the USA.

Underreporting values for human salmonellosis in the different EU MSs were estimated employing updated information on the risk from Swedish travellers in the EU as presented in Table 1 (See Appendix A for detailed calculations). Data were obtained from the Swedish Institute for

Communicable Disease Control (Smittskyddsinstitutet, SMI, Solna, Sweden). The risk of salmonellosis in returning Swedish travellers in the EU was 8.44 per 100,000 travels (90% CI 8.22-8.67), ranging between 0.13 for Finland to 94.26 for Bulgaria. Estimates of the true incidence were anchored to population-based estimates from the Netherlands, based on raw data from a Dutch cohort study (de Wit *et al.*, 2001), where the incidence rates from these studies were applied to the population of 2009 and scaled to the observed average of laboratory-confirmed cases for these years in comparison to the year 1999 when the cohort study was performed. An average of 31,700 (90% CI 6,500-78,600) cases of salmonellosis per year was estimated to occur in the Netherlands in 2009.

**Table 1:** An estimation of the underreporting factor of human salmonellosis in the different EU MSs based on the estimation of the true incidence of human salmonellosis. For detailed explanations on data used, methodology and limitations see Appendix A.

| | **Swedish travellers' Risk** | | **True incidence** | | **Underreporting** | |
|---|---|---|---|---|---|---|
| **Country** | **(per 100,000)** | **(relative to NL)** | **Cases** | **Incidence rate (per 100.000)** | **factor** | **% reported** |
| Austria | 2.92 | 1.899 | 30,483 | 365 | 11.0 | 9.10% |
| Belgium | 0.81 | 0.524 | 10,750 | 101 | 3.5 | 28.96% |
| Bulgaria | 94.26 | 61.303 | 895,981 | 11,779 | 718.5 | 0.14% |
| Cyprus | 23.30 | 15.157 | 23,208 | 2,912 | 173.2 | 0.58% |
| Czech Republic | 23.14 | 15.049 | 302,687 | 2,892 | 28.9 | 3.46% |
| Denmark | 1.35 | 0.879 | 9,303 | 169 | 4.4 | 22.89% |
| Estonia | 2.64 | 1.718 | 4,424 | 330 | 16.9 | 5.90% |
| Finland | 0.13 | 0.085 | 868 | 16 | 0.4 | 268.34% |
| France | 2.39 | 1.554 | 192,097 | 299 | 26.9 | 3.72% |
| Germany | 2.99 | 1.947 | 306,835 | 374 | 9.8 | 10.23% |
| Greece | 35.18 | 22.883 | 495,092 | 4,397 | 1,228.5 | 0.08% |
| Hungary | 31.31 | 20.366 | 392,537 | 3,913 | 66.8 | 1.50% |
| Ireland | 0.32 | 0.211 | 1,803 | 41 | 5.4 | 18.58% |
| Italy | 3.97 | 2.584 | 298,102 | 496 | 71.7 | 1.39% |
| Latvia | 12.46 | 8.107 | 35,223 | 1,558 | 44.3 | 2.26% |
| Lithuania | 29.13 | 18.942 | 121,925 | 3,640 | 59.1 | 1.69% |
| Luxembourg | 1.18 | 0.770 | 730 | 148 | 4.5 | 22.19% |
| Malta | 53.42 | 34.743 | 27,611 | 6,676 | 222.7 | 0.45% |
| Poland | 20.40 | 13.266 | 972,052 | 2,549 | 114.1 | 0.88% |
| Portugal | 34.50 | 22.441 | 458,231 | 4,312 | 2,082.9 | 0.05% |
| Romania | 14.39 | 9.359 | 386,606 | 1,798 | 349.9 | 0.29% |
| Slovakia | 32.89 | 21.389 | 222,436 | 4,110 | 53.2 | 1.88% |
| Slovenia | 9.78 | 6.358 | 24,830 | 1,222 | 40.3 | 2.48% |
| Spain | 16.10 | 10.469 | 921,871 | 2,012 | 214.2 | 0.47% |
| Sweden | NA | 0.085 | 1,508 | 16 | 0.5 | 202.48% |
| The Netherlands | 1.54 | 1.000 | 31,677 | 192 | 26.3 | 3.80% |
| United Kingdom | 1.00 | 0.650 | 76,411 | 125 | 7.3 | 13.71% |
| **EU-27** | **8.44** | **5.490** | **6,245,281** | **1,251** | **57.5** | **1.74%** |
| Norway | 0.24 | 0.159 | 1,464 | 31 | 1.2 | 84.37% |
| Switzerland | 0.98 | 0.639 | 9,456 | 123 | 7.1 | 14.01% |

For the EU-27 the estimated true incidence of salmonellosis is approximately 6 (90% CI 1-15) million cases, which fits well in the range reported before. The underreporting factor at the EU-level is 57.5 (90% CI 11-140). The estimated true incidence rates per MS are visualised in Table 1. Appendix A

describes in detail the data source, methodology used and limitations of the estimated underreporting factors. The estimated underreporting factors were provided to the contractor for their consideration in the model applied to support the quantitative aspects of this Opinion (VOSE, 2011).

These incidence estimates can be used to update a previous estimate of the EFSA Panel on Biological Hazards of the disease burden and costs of salmonellosis, and its sequelae (EFSA, 2010d). The disease burden of salmonellosis and its sequelae is 0.23 (0.05-0.6) million disability-adjusted life years (DALYs) per year and total annual costs are 2 (0.3-4) billion EURO. See Appendix A for further details.

The establishment of active surveillance of human salmonellosis in all Member States, including harmonised typing of human *Salmonella* isolates and efforts to quantify the level of under-ascertainment and underreporting, would improve the estimation of the human health effects of interventions in primary food animal production. This would imply collaborative efforts to enumerate all cases identified in medical microbiological laboratories, so that underreporting is limited as much as possible. Population-based studies to identify the level of under-ascertainment would also be encouraged.

## 3. *Salmonella* in broilers and broiler meat production in the EU

### 3.1. Monitoring of *Salmonella* in broilers

#### 3.1.1. Monitoring systems in the EU

EU Directive 2003/99/EC of the European Parliament and of the council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, provides the legal background for the monitoring of zoonoses in animal populations in Europe. The purpose of this Directive is to ensure that zoonoses, zoonotic agents and related antimicrobial resistance are properly monitored, and that food-borne outbreaks receive proper epidemiological investigation, to enable the collection in the Community of the information necessary to evaluate relevant trends and sources (as reflected in article 1 of the directive).

According to article 4 of the Directive, monitoring shall be based on the systems in place in Member States. However, where necessary to make data easier to compile and compare, detailed rules for the monitoring of zoonoses and zoonotic agents listed in Annex I of the Directive may be laid down.

The first indications on criteria for *Salmonella* monitoring have been laid down in Regulation (EC) 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, which in annex II lists minimum requirements that food business operators have to respect having samples taken and analysed for the control of *Salmonella* in different animal species and categories. As far as broilers are concerned, this Regulation requires all *Salmonella* strains with public health significance to be monitored, through the sampling of birds leaving for slaughter, and specifies that, for a transitional period of three years, the target in broilers shall cover *S*. Enteritidis and *S*. Typhimurium (Article 4 of the Regulation).

Before setting the targets for the reduction of the prevalence of certain *Salmonella* serovars in broiler flocks, a baseline study was organised in all EU member states, and was carried out between October 2005 and September 2006 (Commission Decision 2005/636/EC of 1 September 2005 concerning financial contributions towards a baseline survey on the prevalence of *Salmonella* spp. in broiler flocks of *Gallus gallus* to be carried out in the Member States). For this survey, broiler flocks in holdings containing at least 5,000 birds were sampled within three weeks before slaughter. The number of flocks to be sampled per MS was proportional to the broiler population of the particular MS, and five pairs of boot swabs were collected in each sampled flock.

Following the evaluation of the results of the baseline study, Regulation (EC) 646/2007 set the Community target for the reduction of the prevalence of *S*. Enteritidis and *S*. Typhimurium in broilers

as the reduction of the maximum percentage of flocks of broilers remaining positive for these two serovars combined (i.e. *S.* Enteritidis plus *S.* Typhimurium) to 1 % or less by 31 December 2011. Starting from the 1st January 2009, MSs have to apply *Salmonella* National Control Programmes (NCP) aimed at reaching the target and verifying the expected reduction in prevalence. These NCP must be approved by the European Commission.

In Regulation (EC) 646/2007, the minimum sampling requirements for the verification of target achievement are defined. All flocks of broiler must be included in the National Control Programmes (excluding flocks raised for private domestic use and the ones leading to the direct supply to the final consumer), and that sampling must be carried out:

- as *own checks*, three weeks before the birds are sent to the slaughterhouse;
- as *official controls*, each year in at least one flock of broilers on 10 % of the holdings with more than 5,000 birds.

The samples consist of at least two pairs of boot socks, which must be pooled together for the examination.

Eventually, derogations are foreseen in case of negative results for *S.* Enteritidis and *S.* Typhimurium in the holding for at least one year (plus presence of other farm management conditions on site, as described in Regulation (EC) 646/2007), resulting in the reduction of the frequency of *own checks* to one flock per round on holdings with several flocks.

NCP should consist of proper and effective measures of prevention, detection, and control of *Salmonella* at all relevant stages of the egg and broiler meat production lines, particularly at the level of primary production, in order to reduce *Salmonella* prevalence and the risk to public health. NCP have to be approved by the European Commission, and may largely differ among MSs in the kind of control measures applied (More detailed information on the main characteristics of the national control programmes is available in Appendix B). Results of the programmes as well as any relevant additional information have to be reported to the Commission and EFSA as part of the annual monitoring on trends and sources of zoonoses and zoonotic agents (i.e. published the Community Summary Report on Zoonosis issued annually by EFSA).

According to information reported in the CSR (EFSA, 2011), control measures are applied to all *Salmonella* serovars in eight MSs and only on *S.* Enteritidis and *S.* Typhimurium in twelve MSs. Nine MSs put restrictions on the house immediately after suspicion. Thirteen MS reported that the flock is submitted for sanitary slaughter or heat treated. Four MSs destroy the flock and five MSs carry out slaughtering or destruction depending on the serovar. Cleaning and disinfection of the premises is mandatory in eighteen MSs and restocking can only take place after a negative bacteriological test in sixteen MSs. Only six MSs report to require a specified minimum empty period after disinfection prior to restocking. A number of epidemiological investigations to identify the source of the infection are carried out in most countries with a formal monitoring programme (e.g. trace back in contact flocks, feed suppliers, breeding flocks and hatcheries). Vaccination of broiler parent flocks is permitted in ten MSs and prohibited in four, while vaccination of day-old broiler chicks is not reported.

As a consequence of the application of NCP in MSs, Third Countries (i.e. non-EU countries) have to apply an equivalent programme and have it approved by the European Commission, in order to be included in the list of countries from which MSs can import birds or hatching eggs (Reg. EC 1291/2008).

Therefore, since 1st January 2009 the level of harmonisation in surveillance and control of *Salmonella* in broilers has gradually increased in Member States as well as in Third Countries which export animals to Europe. Before 2009, there was a huge diversity both in monitoring schemes and in control measures among MSs, and this largely hampers the comparability of data reported in the previous

years not only between MSs but also within MSs, due to changes or inconsistencies in the data reported and low coverage of the flocks tested.

Complete harmonisation of the EU statutory monitoring of *Salmonella* in broiler flocks is not expected to be fully accomplished since the *Salmonella* NCP may vary with regard to sampling methods and frequency even if the minimum requirements for the monitoring are fulfilled. Currently, some MSs carry out more intensive programmes (e.g. more samples taken and/or sampling more than once in the broiler production period) than required by the statutory monitoring, which may also impact the reported results and consequently the comparability between MSs. On the other hand, the level of detail (at least serovar detail) in the data reported varies between MSs. For the 2009 monitoring, 10 out of the 27 EU MSs report only serovar details for *S.* Enteritidis and *S.* Typhimurium. Overall, no precise serovar information is available for 52% of the reported positive flocks in the EU in 2009.

### 3.1.2. Results of monitoring

Figure 1 shows individual MS prevalence results (% of positive broiler flocks tested) of *S.* Enteritidis and *S.* Typhimurium combined of both the 2009 EU statutory monitoring and the baseline survey in broiler flocks carried out in the EU between 2005 and 2006 in the different EU MS.

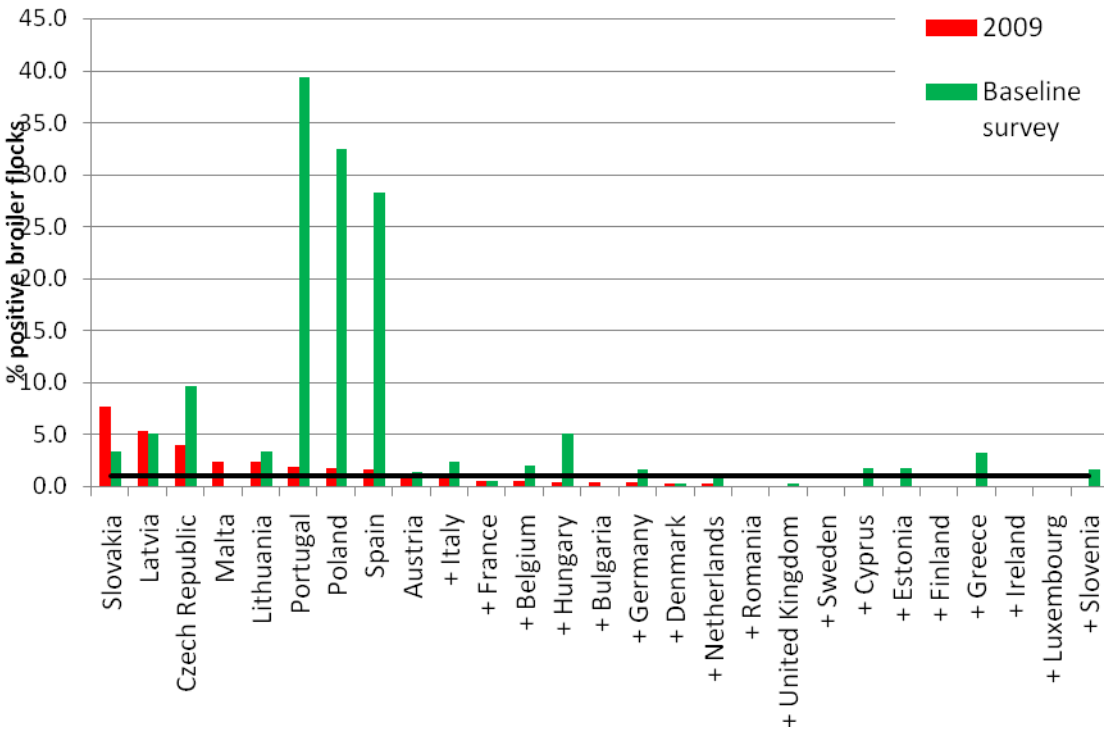



**Figure 1:** Prevalence of *S.* Enteritidis and *S.* Typhimurium in broiler flocks combined in the different EU MSs as reported through the 2009 statutory monitoring and through the baseline survey in broiler flocks carried out between 2005 and 2006. The black line represents the EU target for 2010 (1% positive flocks for *S.* Enteritidis and *S.* Typhimurium combined). MSs of which the name is preceded by (+) symbol have met the target in 2009.

Results of the 2009 EU statutory monitoring show that already 18 MSs met the target of 1% or less flocks positive to *S.* Enteritidis and/or *S.* Typhimurium. Seven of those 18 MSs reported no finding of the two serovars at all.

Figure 2 shows individual MS prevalence results (% of positive broiler flocks tested) of all *Salmonella* spp. both for the 2009 EU statutory monitoring and through the baseline survey in broiler flocks carried out in the EU between 2005 and 2006 in the different EU MS.

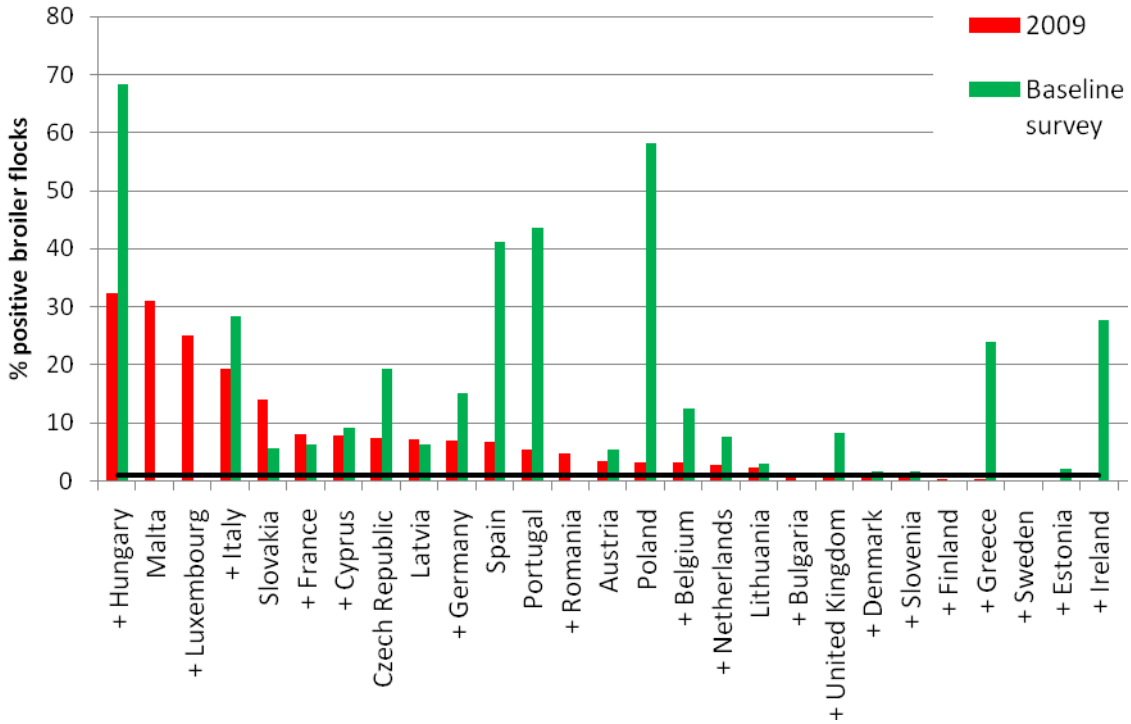



**Figure 2:** Prevalence of *Salmonella* spp. in broiler flocks in the different EU MSs as reported through the 2009 EU statutory monitoring and the baseline survey in broiler flocks carried out between 2005 and 2006. The black line represents the EU target for 2010 (1% positive flocks for *S.* Enteritidis and *S.* Typhimurium combined). MSs of which the name is preceded by (+) symbol have met the target in 2009.

Results of the 2009 EU statutory monitoring show that already 7 MSs reported a *Salmonella* spp. prevalence for all serovars combined below 1%. In 2 MSs, no *Salmonella* spp. positive flocks were reported at all.

Table 2 shows results of the EU mandatory monitoring in 2009 in broiler flocks before slaughter, from the baseline survey in broiler flocks carried out between 2005 and 2006 and from the baseline survey of *Salmonella* in broiler carcases carried out in 2008.

**Table 2:** Results of the EU monitoring of *Salmonella* in adult broiler flocks for 2009 (harmonised monitoring), from the Baseline Survey carried out in adult broiler flocks between 2005 and 2006 and from the Baseline Survey carried out in broiler carcasses in 2008.

| | 2009 Monitoring | | | BSL Flocks 2005-2006[1] | | | BSL Carcasses 2008[2] | | |
|---|---|---|---|---|---|---|---|---|---|
| | N | % pos (all) | % S. Enteritidis and S. Typhimurium | N | % pos (all) | % S. Enteritidis and S. Typhimurium | N | % pos (all) | % S. Enteritidis and S. Typhimurium |
| Austria | 3,302 | 3.4 | 1.1 | 365 | 5.4 | 1.3 | 408 | 2.5 | 0.7 |
| Belgium | 8,049 | 3.1 | 0.5 | 373 | 12.4 | 2 | 380 | 16.3 | 2.9 |
| Bulgaria | 1,152 | 1.4 | 0.4 | - | - | - | 316 | 22.5 | 6 |
| Cyprus | 239 | 7.9 | 0 | 248 | 9.1 | 1.7 | 357 | 10.6 | 0 |
| Czech Republic | 6,035 | 7.4 | 4 | 334 | 19.3 | 9.6 | 422 | 5.5 | 1 |
| Denmark | 3,767 | 0.9 | 0.3 | 295 | 1.6 | 0.3 | 396 | 0.0 | 0 |
| Estonia | 414 | 0 | 0 | 139 | 2 | 1.7 | 102 | 0.0 | 0 |
| Finland | 2,972 | 0.4 | 0 | 360 | 0.1 | 0 | 369 | 0.0 | 0 |
| France | 35,913 | 8.1 | 0.5 | 381 | 6.2 | 0.5 | 422 | 7.6 | 0.2 |
| Germany | 4,339 | 7 | 0.4 | 377 | 15 | 1.6 | 432 | 15.0 | 4.6 |
| Greece | 6,577 | 0.3 | 0 | 245 | 24 | 3.2 | - | - | - |
| Hungary | 4,491 | 32.4 | 0.4 | 359 | 68.2 | 5.1 | 321 | 88.5 | 4.4 |
| Ireland | 665 | 0 | 0 | 351 | 27.6 | 0 | 394 | 9.9 | 0 |
| Italy | 2,072 | 19.2 | 1 | 313 | 28.3 | 2.3 | 393 | 13.5 | 0.3 |
| Latvia | 566 | 7.1 | 5.3 | 121 | 6.2 | 5.1 | 122 | 4.9 | 4.9 |
| Lithuania | 218 | 2.3 | 2.3 | 156 | 2.9 | 3.3 | 374 | 2.1 | 0.3 |
| Luxembourg | 4 | 25 | 0 | - | - | - | 13 | 0.0 | 0 |
| Malta | 87 | 31 | 2.3 | - | - | - | 367 | 15.0 | 0 |
| Netherlands | 29,193 | 2.7 | 0.2 | 362 | 7.5 | 1 | 429 | 9.6 | 0.2 |
| Poland | 20,665 | 3.2 | 1.7 | 357 | 58.2 | 32.4 | 419 | 25.5 | 9.6 |
| Portugal | 654 | 5.4 | 1.8 | 367 | 43.5 | 39.3 | 421 | 11.2 | 9 |
| Romania | 3,160 | 4.8 | <0.1 | - | - | - | 357 | 4.8 | 0.8 |
| Slovakia | 544 | 14 | 7.7 | 230 | 5.7 | 3.3 | 422 | 21.6 | 6.4 |
| Slovenia | 3,080 | 0.7 | 0 | 326 | 1.6 | 1.6 | 413 | 1.7 | 0.5 |
| Spain | 13,620 | 6.7 | 1.6 | 388 | 41.2 | 28.2 | 389 | 14.9 | 6.7 |
| Sweden | 2,713 | 0.1 | <0.1 | 291 | 0 | 0 | 410 | 0.2 | 0 |
| United Kingdom | 27,780 | 1.3 | <0.1 | 382 | 8.2 | 0.2 | 401 | 3.2 | 0 |
| **EU Total** | **182,271** | **5.0** | **0.7** | **7,120** | **23.7** | **11.0** | **9,249** | **12.2** | **2.3** |

1 Broiler flock prevalence estimate. For details see reference original EFSA Scientific Report (EFSA, 2007a).

2 Calculations based on raw data. These may slightly differ from estimates presented in original EFSA Scientific Report (EFSA, 2010a).

Overall, the results show a very large reduction in the combined prevalence of *S*. Enteritidis and *S*. Typhimurium in the three MSs with the highest prevalence (Figure 1 and Table 2) as well as in all *Salmonella* serovars (Figure 2 and Table 2).

The observed reductions are undoubtedly achieved by hard work through the EU control and monitoring programmes of *Salmonella* in poultry (*Gallus gallus*). In particular, the effect of the *Salmonella* NCPs in breeding hens, where EU-wide targets for specific *Salmonella* serovars (including *S.* Enteritidis and *S.* Typhimurium) have been in place since 2007, is considered to have contributed significantly to the observed reduction of the *Salmonella* prevalence in the production lines.

However, as described in the following section other factors may have contributed to a lower sensitivity of the monitoring program in broiler flocks when compared to the baseline survey carried out in flocks in 2005-2006. The results from the baseline survey and the reported through the NCPs may therefore not be directly comparable for all MSs.

### 3.1.3. Factors influencing the detection of *Salmonella* in broiler flocks

Sampling methodologies have a big impact on estimates of prevalence and evaluation of the effectiveness of control measures (Chriél et al, 1999; Fletcher 2006). This applies to farms, feedmills and hatcheries (Bailey et al, 1999; Rocha *et al* 2003; Chao et al, 2007). The diligence of the sampler in taking a representative sample is critically important.

Multiple bootswab samples identify more positive flocks than less intensive sampling methods such as a more limited number of pooled faecal/bootswab samples or cloacal swabs (Skov et al 1999b and Heyndrickx et al 2002). The sensitivity of the sampling carried out in the EU baseline survey of *Salmonella* in broiler flocks decreased with a lower within-flock sample size. On average over the simulations carried out in the study, the calculated EU flock prevalence decreased from 11% when 5 samples were taken per flock to 9% based on 2 samples per flock and 7% with only one sample per flock. At the individual MS level, the general tendency follows a similar pattern. However, variability across MSs was very large being this linked to both the (diagnostic and analytical) test sensitivity and the within-flock prevalence. The more sensitive the test and the higher and more homogenous the level of *Salmonella* is in the flock, the less the estimated flock prevalence will decrease when taking fewer samples. What is less easy to estimate is the effect of pooling the two pairs of boot swabs from the whole house that are taken in the monitoring programme into one pooled sample for testing and how this single pooled sample test compares with the results of the simulation study carried out by EFSA, which considered pairs of bootswabs that were each taken in one fifth of the house only. It is likely however that the reduction of sampling from five pairs of boot swabs tested as individual pairs, as done in the baseline survey, to two pairs tested as one pool in the EU harmonised monitoring programme is likely to result in a significantly lower sensitivity and thereby detected flock prevalence. This means that a reduction in the MSs flock prevalence as reported by the EU statutory harmonised monitoring programme compared to the flock prevalence found in the baseline survey may at least to some extent be explained by a decreased sampling sensitivity depending on the NCP actually carried out in the different MSs (EFSA, 2007c). Although the EU statutory monitoring in the MSs is likely to have a lower sensitivity resulting in a larger estimated reduction, it should be noted that the individual MSs's contributions to this reduction varies greatly.

The sampling material may also influence the sampling sensitivity. Inclusion of dust samples in monitoring programmes also helps to identify infected flocks more effectively (Davies 2004). The sensitivity of sampling can also be influenced by inhibitory substances added to litter (Bennett et al, 2003; Garrido et al, 2004), addition of new litter before sampling, and litter moisture (Carr *et al* 1995; Mallinson et al, 2000 and Myint et al, 2005).

Regular failures to detect *Salmonella* in broiler houses where there is low within-flock prevalence may create a mistaken impression of sporadic introduction of infection whereas in reality the house may be continuously contaminated. Sampling practices (i.e. surface and areas of the house covered by the bootswab sampling) can also influence the likelihood of detecting a positive flock, in particular in flocks with low within flock prevalence.

Once a sample is taken, there are many factors relating to transit and storage conditions as well as correct application of laboratory testing methodology which can compromise the detection of *Salmonella* from positive samples (Carrique-Mas and Davies, 2008; Davies and Wray, 1997b).

In summary, there are many factors that may influence the final test result. Flocks that are highly infected in terms of within-flock prevalence and numbers of organisms excreted are however likely to be most easily detected, and this may also reflect the public health risk associated with meat products derived from the flock. It must therefore be anticipated that flocks will remain undetected and that the proportion of undetected flocks will vary between MSs.

The most accurate way to assess the progress on *Salmonella* control implemented in broiler flocks in all Member States would be to repeat the baseline survey performed in 2005 and 2006. By doing this, different biases or inconsistencies between the monitoring performed throughout the different years could be reduced. These include, for example, the possible influence of sampling technique and variability between commercial laboratories, and the differences between MSs in the level of serotyping carried out and reported.

### 3.2. Monitoring of *Salmonella* in broiler meat

Monitoring of *Salmonella* in different types of broiler meat and products thereof in the EU is mainly carried out in the context of ensuring compliance with microbiological criteria (both food safety criteria for products and process hygiene criteria for broiler carcasses). Earlier Scientific Opinions of the Scientific Panel on Biological Hazards have addressed and considered in detail microbiological criteria issues for poultry meat (including broiler meat from *Gallus gallus*) in the EU (EFSA, 2007a, 2010e). The latest of these Opinions, on the link between *Salmonella* criteria at different stages of the poultry production chain replied to a request from the European Commission in the frame of the possible establishment of a food safety criteria for *Salmonella* in fresh poultry meat.

The reporting of *Salmonella* in broiler meat and products thereof in the EU presents several limitations when comparing and interpreting the results of monitoring between different MSs (EFSA, 2010e; EFSA and ECDC, 2011), namely:

- Differences in test and analytical sensitivity from the different monitoring in the EU MSs.
- Monitoring of *Salmonella* in broiler meat is not fully harmonised between MSs. Sampling composition and size and sampling frequency varies between MSs. Also, the stage at which samples are taken during production may vary.
- The level of detail of reporting provided by the MSs differs.
- Detailed distribution of *Salmonella* serovars in broiler meat is not consistently presented (10 MSs in 2009).

At slaughter, the reported proportion of positive samples in 2009 varied among MSs from 0% to 60.8%, while at processing *Salmonella* was detected in 0% to 31.1% of the samples(EFSA and ECDC, 2011). For the MSs that reported findings of *Salmonella* at retail, results ranged from 0% prevalence to 36.1%.

A baseline survey was carried out in 2008 in the EU on the prevalence of *Campylobacter* and *Salmonella* in broiler carcases (EFSA, 2010a). The sampling of broiler carcasses was based on a random selection of slaughterhouses, sampling days in each month and the batches to be sampled on each day. The randomisation scheme aimed at selecting broiler batches proportionate to the number of broiler flocks, fattened according to the different production types. Results of this baseline survey can be found in Table 2.

A useful exercise done in order to get an overview of the comparability of the reported data on *Salmonella* in broiler meat production at different stages and sampled under different sampling schemes has being previously presented by EFSA (EFSA, 2010e). Results showed variability between MSs when comparing the results obtained in the different monitoring and testing schemes. While in some MSs the isolation of *S.* Enteritidis and/or *S.* Typhimurium in both poultry flocks and poultry meat was observed, for others this is not the case (e.g. serovars isolated from broiler carcasses in some MSs but not from the broiler flock population).

### 3.3. Concluding remarks on monitoring of *Salmonella* in broiler meat production in the EU

Regarding the monitoring of *Salmonella* throughout the broiler meat production chain, the following concluding remarks can be made:

- Due to the between-years and between-MSs differences in the EU monitoring schemes from *Salmonella* in broiler flocks, it is not possible to accurately assess the progress done in reducing *Salmonella* in broiler flocks.

- Complete harmonisation of the EU statutory monitoring of *Salmonella* in broiler flocks is not expected to be fully accomplished since the National Control Programmes may vary with regard to sampling methods and frequency even if the minimum requirements for the monitoring are fulfilled.

- The most accurate way to assess the progress on *Salmonella* control implemented in broiler flocks in all Member States would be to repeat the baseline survey performed in 2005 and 2006. By doing this different biases or inconsistencies between the monitoring performed throughout the different years could be avoided.

- Currently, monitoring of *Salmonella* in broiler meat is not fully harmonised.

## 4. *Salmonella* serovars of public health significance.

As previously addressed by EFSA, any serovar that is not animal host-specific is considered capable of causing gastro-intestinal illness of varying severity in humans, and thus should be considered of potential public health significance (EFSA, 2004, 2009, 2010d). Nevertheless, temporal associations between some serovars and particular animal reservoir may occur, which impact the frequency of human illness and consequently may affect food safety decision making.

Currently the genus *Salmonella* is divided into two species: *S. enterica* and *S. bongori*. The species *S. enterica* consist of six subspecies*: enterica*, *salamae*, *arizonae, diarizonae, houtenae* and *indica*, whereas no subspecies have been assigned to *S. bongori* (Su and Chiu, 2007). Within the species and subspecies, more than 2,600 different serovars have been identified (Guibourdenche et al., 2010). The vast majority of zoonotic serovars associate d with human illness belong to *S. enterica* ssp. *enterica*.

Almost all *Salmonella* serovars are considered to be potentially pathogenic for humans, but the degree of host adaptation varies, which affects the pathogenicity. Some serovars: e.g. *S.* Typhi, *S.* Paratyphi and *S.* Sendai, are specific to man (Molbak et al., 2006). They cause severe systemic illness in humans characterised by fever and abdominal symptoms (enteric/(para)typhoid fever) (Miller et al., 1995). These serovars are usually non-pathogenic in animals and are not considered to have a zoonotic potential.

Non-typhoidal, ubiquitous serovars, such as *S.* Typhimurium and *S.* Infantis, affect a wide range of animals and humans. Although such serovars in principle are non-host-adapted, strong associations between certain serovars or subtypes within a serovar and a given animal reservoir may occur, like for example *S.* Enteritidis of various phage types in laying hens and *S.* Enteritidis PT11 in hedgehogs. In contrast, there exists a group of serovars that are highly adapted to an animal host (e.g. *S.* Choleraesuis in pigs, *S.* Dublin in cattle, *S.* Abortusovis in sheep, and *S.* Gallinarum in poultry). These serovars only

occasionally infect humans, where they may produce no, mild or serious disease according to the specific serovar (Acha and Szyfres, 2001; Molbak et al., 2006). The non-host-adapted serovars are those with principal zoonotic significance and the ability of these to infect animals and eventually infect humans via contamination of food seems to vary (Hald et al., 2006; Pires et al., 2010b))(Pires et al., 2010a).

From a regulatory point of view, and as per the guidance provided in the Terms of Reference of the request made by the Commission, criteria for *Salmonella* monitoring have been laid down in Regulation (EC) No. 2160/2003. Annex II of this Regulation lists minimum requirements that food business operators have to respect by having samples taken and analysed for the control of *Salmonella* in different animal species and categories. As far as flocks of *Gallus gallus*, turkeys and pigs are concerned, the Regulation requires all *Salmonella* serovars with public health significance to be monitored, at different production stages. Annex III of Reg. 2160/2003 defines the specific criteria to be adopted to determine *Salmonella* serovars with public health significance to which community targets will apply. These are based on the following factors:

- the most frequent *Salmonella* serovars in human salmonellosis on the basis of data collected through EC monitoring systems;
- the route of infection (that is, the presence of the serovar in relevant animal populations and feed);
- whether any serovar shows a rapid and recent ability to spread and to cause disease in humans and/or animals;
- whether any serovar show increased virulence, for instance as regards invasiveness, or resistance to relevant therapies for human infections.

In this Opinion, available information and data will be used to address these criteria in relation to broilers and broiler products hereof.

### 4.1. *Salmonella* serovars in human salmonellosis

The ranking of the serovars most frequently isolated from reported cases of human salmonellosis cases in European countries for 2006 - 2009, is presented in Table 3. The reported human data represent aggregated data and in some cases serovars reported individually in one year may be reported in the group “Other” in previous years.

**Table 3:** *Salmonella* serovars reported from confirmed salmonellosis cases in humans in the EU (based on EFSA and ECDC, 2011, 2010 and 2009).

| | Reporting year | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2009[1] | | 2008[1] | | 2007[1] | | 2006[2] | |
| **Serovar** | **N** | **%** | **N** | **%** | **N** | **%** | **N** | **%** |
| *S*. Enteritidis | 53,382 | 52.3 | 70,091 | 58 | 81,472 | 64.5 | 90,362 | 71 |
| *S*. Typhimurium | 23,759 | 23.3 | 26,423 | 21.9 | 20,781 | 16.5 | 18,685 | 14.7 |
| *S*. Infantis | 1,616 | 1.6 | 1,317 | 1.1 | 1,310 | 1 | 1,246 | 1 |
| *S*. Bovismorbificans | 433 | 0.4 | 501 | 0.4 | np | np | np | np |
| *S*. Hadar | 507 | 0.5 | np | np | 479 | 0.4 | 713 | 0.6 |
| *S*. Virchow | 736 | 0.7 | 860 | 0.7 | 1,068 | 0.8 | 1,056 | 0.8 |
| *S*. Derby | 671 | 0.7 | 624 | 0.5 | 469 | 0.4 | 477 | 0.4 |
| *S*. Newport | 760 | 0.7 | 787 | 0.7 | 733 | 0.6 | 730 | 0.6 |
| *S*. Stanley | np | np | 529 | 0.4 | 589 | 0.5 | 522 | 0.4 |
| *S*. Agona | np | np | 636 | 0.5 | 387 | 0.3 | 367 | 0.3 |
| *S*. Kentucky | 460 | 0.5 | 497 | 0.4 | 431 | 0.3 | 357 | 0.3 |
| *S*. Saintpaul | 452 | 0.4 | np | np | np | np | np | np |
| Other | 19,225 | 18.8 | 18,495 | 15.3 | 18,562 | 14.7 | 12,790 | 10 |
| Total | 102,001 | | 120,760 | | 126,281 | | 127,305 | |
| Unknown | 6,613 | | 6,636 | | 9,814 | | 17,359 | |

np = Not presented in the published data.

[1] Source: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden and United Kingdom.

[2] Source: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Luxembourg, Malta, the Netherlands, Portugal, Slovakia, Slovenia, Spain, Sweden and the United Kingdom.

Data in Table 3 show that in the EU-total in the period 2006 to 2009, *S*. Enteritidis or *S*. Typhimurium were found in at least 75% of all reported cases of human salmonellosis where the isolate was serotyped. It has to be noted that the number of *S*. Enteritidis associated reported cases has downward trend since 2006 at an increased rate, with an approximate 10% decrease from 2006 to 2007 to an approximate 20% reduction between 2008 and 2009. In contrast, reported cases of S. Typhimurium increased with around 40% from 2006 to 2008, where it decreased with 18% in 2009. The other three serovars for which targets in breeding hens of *Gallus gallus* are set according to Regulation (EC) No 1003/2005 (*S*. Hadar, *S*. Infantis and *S*. Virchow) together were associated with approximately 2-3% of cases and *S*. Hadar is no longer amongst the 'top 5' serovars in humans, having been displaced by *S*. Newport. Around 10-20% of cases were associated with a variety of other serovars, none of them individually exceeding 1%. Between 6,613 and 17,359 *Salmonella* isolates were of "unknown" serovar. This group includes untyped isolates, where no typing was attempted and untypeable isolates, where typing was attempted but outcome was not successful.

The reduction in the number of human salmonellosis cases due to *S*. Enteritidis is believed to be a result of an improved surveillance and control of *S*. Enteritidis in breeding hens and in laying hens in many MSs (EFSA, 2009, 2010d). Controlling the occurrence of this serovar in breeding flocks would impair its vertical transmission to production lines (i.e. laying hens and broilers). On the other hand,

controlling the occurrence of this serovar during production in laying hens would control its presence in eggs, which are considered the main exposure route to this serovar for human salmonellosis. In contrast, the increased reported incidence of *S.* Typhimurium infections may indicate that one or more sources of these infections are increasing in importance.

It should be noted that monophasic variants of *Salmonella* Typhimurium-like strains appear to be of increasing importance in many EU MSs (EFSA, 2010h). In the EFSA Scientific Opinion on this topic, it was concluded that the public health risk posed by these emerging monophasic *S.* Typhimurium strains is considered comparable to that of other *S.* Typhimurium strains which have caused widespread epidemics of infection over the past four decades.

### 4.2. *Salmonella* serovars in the broiler meat production chain

There is no doubt that most strains of *S.* Enteritidis and *S.* Typhimurium (apart from a few phage types that are host adapted to wildlife species) that can infect broiler flocks are also of potential public health significance even though other sources of these strains such as eggs, pig meat, beef, milk products or companion animals may present a greater risk (Berghold et al., 2004; Poirier et al., 2008).

The recent emergencing dissemination of monophasic variants of *S.* Typhimurium-like strains (i.e. 1, 4,[5],12:i:- ) in pigs, cattle, companion animals and humans represents a new threat for broiler production in the EU as the environmental load of this organism, and the associated risk of contamination of feed ingredients and breaches in biosecurity increases. Although no cases of this organism were reported in the Baseline Survey of broiler flocks, this was carried out in 2005 and 2006 before the widespread appearance of monophasic variants of *S.* Typhimurium-like strains in pigs in 2007. Despite monophasic variants of *S.* Typhimurium-like strains isolates not being found in broiler at farm level, they were reported in the baseline study of broiler carcases conducted in 2008 (EFSA, 2010a), which might indicate the more recent emergence of monophasic *S.* Typhimurium in broiler production. *S.* 1, 4,[5],12:i:- monophasic strains of *S.* Typhimurium were reported from 15 of 1,225 *Salmonella*-positive broiler carcases in 4 countries (Germany, Malta, the Netherlands and Switzerland). A small number of poultry cases have also been identified in the UK in 2009. The cause of the simultaneous worldwide emergence of multiple clonal lines of monophasic *S.* Typhimurium lacking the *flj*B gene that encodes the phase two flagella antigens h:1,2 during the last decade is unknown, but the prominence of such strains amongst human cases indicates a significant public health threat (EFSA, 2010g) and new EU controls may be introduced to ensure that such strains are treated as *S.* Typhimurium for regulatory purposes.

For the 2009 EU statutory monitoring, 10 out of the 27 EU MSs report only serovar details for *S.* Enteritidis and *S.* Typhimurium, and for 51% of the reported positive flocks in the EU no serovar name is reported. The most detailed and recent EU-harmonised serovar information for *Salmonella* in broiler meat production can be retrieved from the EU Baseline Survey carried out in broiler carcasses in 2008. In Figure 3, the results of the distribution of the top 5 serovars of the positive batches found in this baseline survey for the different MSs is compared with the serovar distribution for the same MS where positive flocks were found in the baseline survey on *Salmonella* in broiler flocks, the later carried out in 2005-2006. Results are shown only for MSs that provided positive results in both baseline surveys.

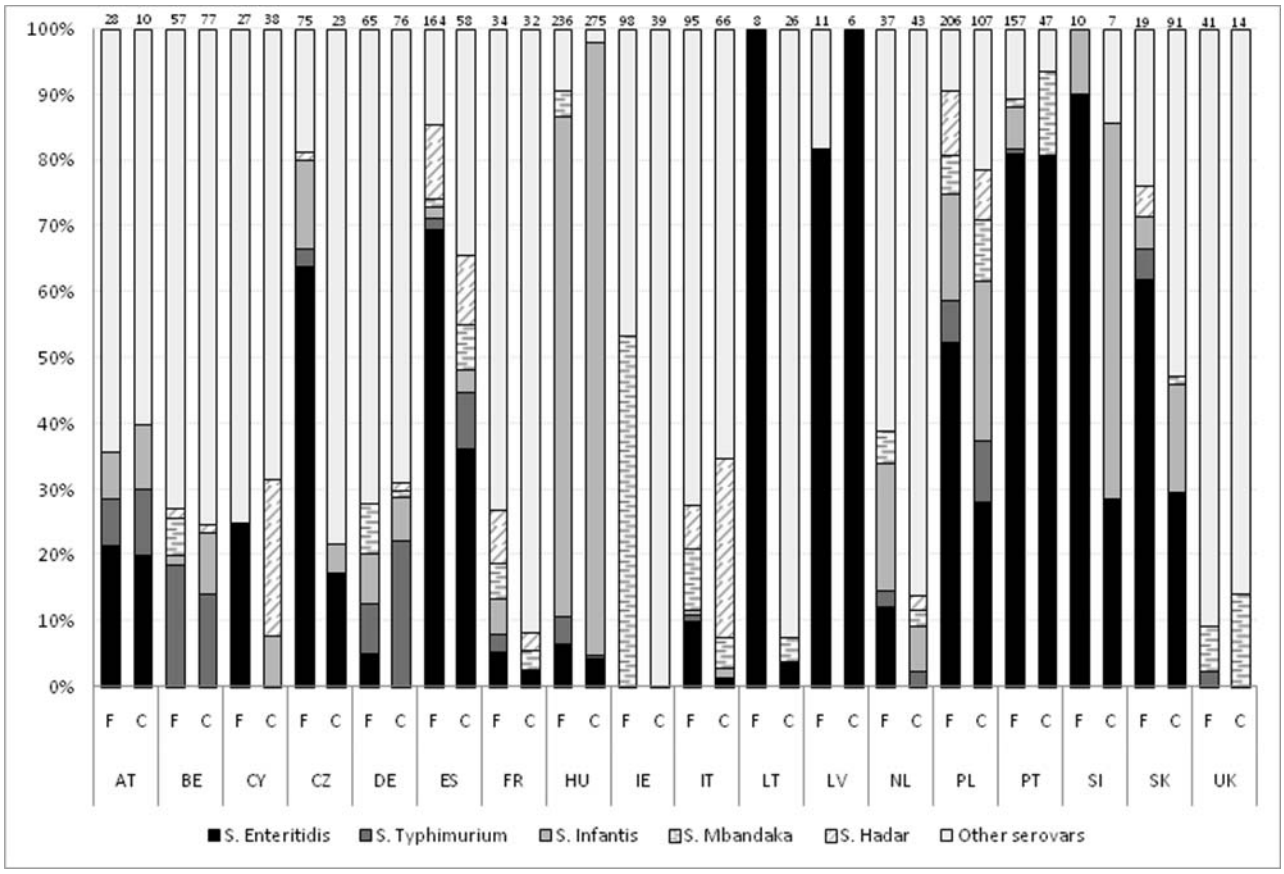



**Figure 3:** Relative distribution of *S.* Enteritidis, *S.* Typhimurium, *S.* Infantis, , *S.* Mbandaka, and *S.* Hadar in the baseline survey of broiler flocks (F) and the baseline survey of broiler carcasses (C) in 18 MSs. The number at the top of each bar represents the total number of sampled units positive in each of the relevant baseline surveys. Results are shown only for MSs that provided positive results in both baseline surveys. Figure provided by the EFSA Unit on Zoonoses data collection.

In Figure 4, the results of the distribution of the frequency of the top 5 serovars found in human salmonellosis in 2009 are presented from the baseline survey for the different MSs in both baseline surveys in broiler flocks and broiler carcasses. Results are shown only for MSs that provided positive results in both baseline surveys.

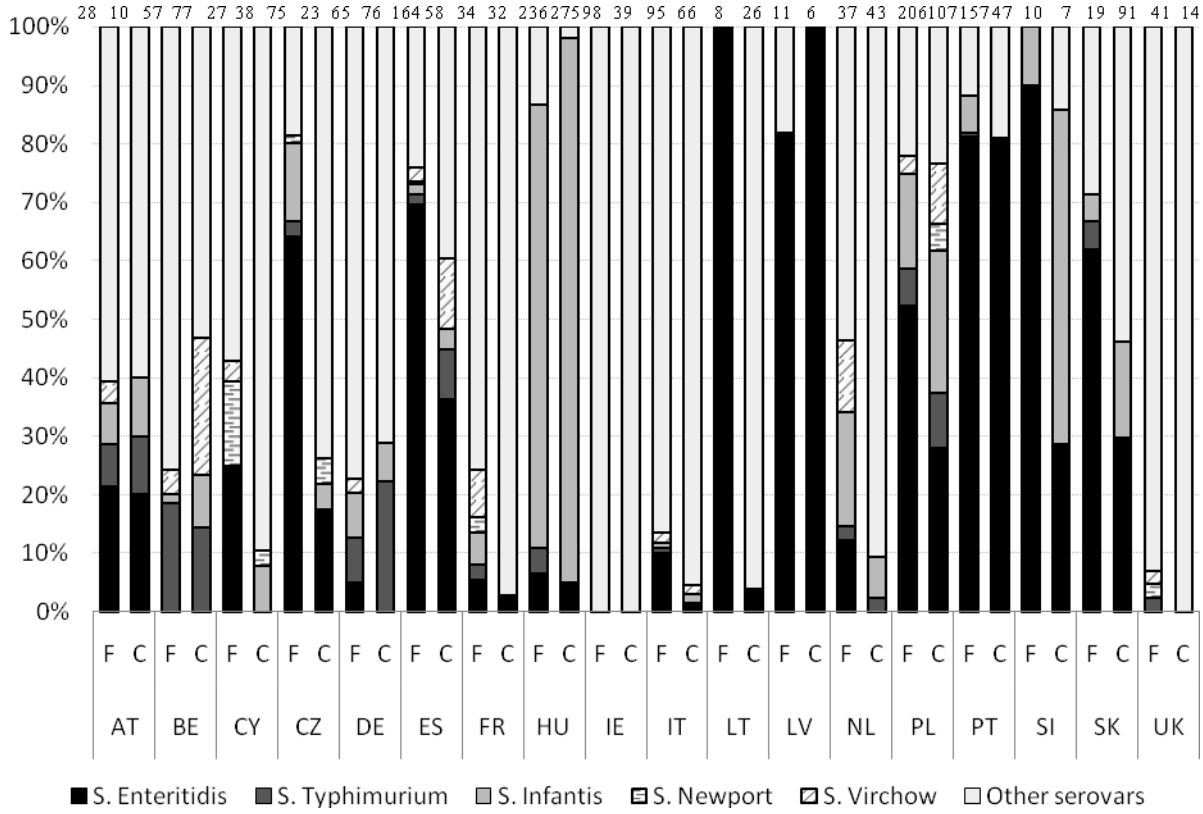



**Figure 4:** Relative distribution of *S.* Enteritidis, *S.* Typhimurium, *S.* Infantis, *S.* Newport, and *S.* Virchow in the baseline survey of broiler flocks (F) and the baseline survey of broiler carcasses (C) in 18 MSs. The number at the top of each bar represents the total number of sampled units positive in each of the relevant baseline surveys. Results are shown only for MSs that provided positive results in both baseline surveys. Figure provided by the EFSA Unit on Zoonoses data collection.

The baseline surveys showed that many of the most frequently found serovars were found in both baseline surveys in most MSs. Differences in the serovar distribution between the two studies may be explained by the fact that the two studies were done two years apart, in which period targeted control of specific serovars in both breeding and production flocks is likely to have shifted the serovar distribution. Introduction of new serovars through feed or breeding stocks may also have contributed to this difference.

The current National Control Programmes on breeding hens and production flocks appear to have already achieved a significant reduction in the number of broiler-associated human cases. Further improvements are mainly to be achieved by focussing on continuous reduction in the prevalence of particular *S.* Enteritidis in broiler breeding (EFSA, 2009) and production. It is underlined, however, that targeting specific serovars very strictly may create a niche for other and possible emerging serovars and could result in a relative or absolute increase in the occurrence of such serovars or strains. New zoonotic Salmonellae, such as the monophasic variants of *S.* Typhimurium-like strains, may arise unpredictably and there is a need for mechanisms to identify the emergence of such strains at an early stage.

Finally, it has to be noted that many *Salmonella* serovars that are found in broiler feed production, hatcheries or broiler farms are not found regularly on poultry meat or in humans (Baumgartner et al., 1992; Corry et al., 2002; Gutierrez et al., 2009; Huehn and Malorny, 2009; Pelkonen et al., 1994; Snow et al., 2008; Sumner et al., 2004), but strains of non- *S.* Enteritidis and/or *S.* Typhimurium serovars may become more prominent and constitute a zoonotic risk (Nogrady et al., 2007; Olsen et al., 1992) following breaches in slaughter hygiene, improper handling, cooking or cooling of meat

(Moore et al., 2003; Oscar, 2004b). In addition, international trade of broiler meat may expose new susceptible populations to *Salmonella* strains that they do not regularly encounter in domestic products. This may lead to a higher prevalence of infection (Threlfall et al., 2005).

### 4.3. Changes in the ability of different *Salmonella* serovars to spread and cause disease in humans and animals

As discussed above some changes in the ranking of the frequencies of *Salmonella* serovars involved in human disease have been occurring in the EU as a whole since Regulation (EC) 1003/2003 came into force. For instance, *S*. Hadar has not been among the top 5 in the last two reported years, and the two most frequent serovars are still *S*. Enteritidis and *S*. Typhimurium, they show opposite trends (see Table 3).

Besides this, there are trends in the frequencies of isolated *Salmonella* serovars in some countries, although these may not influence the overall ranking amongst human infections at EU level. One example is the emergence and dominance of multidrug-resistant clone of *S*. Infantis in broilers, which has been accompanied by an increased prevalence of this serovar in the human population in Hungary (Nogrady et al., 2007). An increased prevalence of *S*. Infantis has also been reported in broilers in Poland (EFSA, 2007d) and in Israel, where it contributed to 34% of human cases (Gal-Mor et al., 2010).

Another example is the increasing prominence of the multidrug-resistant *S*. Paratyphi B variant Java in human outbreaks in several EU MSs (Denny et al., 2007). This serovar has been isolated with high prevalence from poultry and poultry products in Germany and the Netherlands (Dorn et al., 2001; Miko et al., 2002). In German broiler flocks the monophasic *Salmonella enterica* subsp. *enterica* serovar 4,12:d:- was the most frequently isolated serovar (prevalence of 23.6%). In Denmark and the United Kingdom, its serovar prevalence was 15.2% and 2.8%, respectively. Although poultry is a major source of human salmonellosis, this serovar 4,12:d:- seems to be one of the exceptions. It is rarely isolated in humans (approximately 0.09% of human cases per year). This may be explained by DNA microarray analysis results which show that the serovar is highly clonal and lacks genes with known contributions to pathogenicity, and antimicrobial resistance (Huehn et al., 2009). Recent data from the Republic of Ireland report the dominance of *S*. Mbandaka and *S*. Kentucky in broiler flocks, although an epidemiological link between the animal and human strains could not be established (Gutierrez et al., 2009).

### 4.4. Increased virulence or resistance to relevant therapies for human salmonellosis

In general, the course of non-typhoidal salmonellosis in humans is characterised by a clinical picture which may include fever, diarrhoea, abdominal pain, nausea and vomiting. Hospital admission may sometimes be required. In infants and young children, in the elderly and immunologically suppressed, some fatal cases may occur (ECDC, 2008). Direct causal mortality rates are still largely unknown. Available human data for the year 2009 are discussed in chapter 2. Definitive evidence for increased virulence of specific *Salmonella* serovars or of subtypes or clones for humans is very difficult to obtain.

For *S*. Enteritidis, and in particular phage types (PTs) 1, 4, 6, 8 and 14b in poultry, there are several epidemiological and clinical data from the past indicating an increased ability to colonize the oviduct of breeders and layers, and to spread vertically through breeding pyramids (Humphrey, 2006; Humphrey et al., 1989; Mizumoto et al., 2005; Poppe, 2000; Turcotte and Woodward, 1993). In general, it is the ability of some *Salmonella* serovars, or individual strains within serovars for vertical transmission that determines their importance in breeding flocks (EFSA, 2009, 2010d).

One recent study reports that *Salmonella* serovars that are closely related genetically may differ significantly in their pathogenic potential for man (Jones et al., 2008). A significantly increased mortality up to one year after infection with some Multiple Drug-Resistant (MDR) zoonotic *S*.

Typhimurium definitive phage type (DT) 104 has been observed in Denmark in the past (Helms et al., 2003).

The emergence of MRD *Salmonella* types e.g. *S.* Typhimurium DT 104 and more recently the monophasic variants of *S.* Typhimurium-like strains (*S.* 1,4,[5],12:i;-) strains (EFSA, 2010g), is of special concern in humans with pre-infection antimicrobial therapy for indications other than gastroenteritis. The use of antimicrobials for food animals is a contributing factor for the selection and dissemination of drug-resistant *Salmonella* (Emborg et al., 2008; van den Bogaard and Stobberingh, 1999). The increasing use of antimicrobials, particularly fluoroquinolones, in humans has recently also been shown to be associated with an increased incidence of infections caused by drug-resistant *Salmonella* (Koningstein et al., 2010).

Although the hypothesis of increased pathogenicity of *S*. Typhimurium DT104 for different animal species is still unconfirmed, resistance to therapies due to frequent MDR of this serovar is considered to be an important advantage of this pathogen for survival and spread in animals and man (Helms et al., 2005; Szmolleny et al., 2000). Some studies in the 1990s suggested that compared with patients infected with susceptible *Salmonella* strains, patients with MDR *S*. Typhimurium DT104 infections were more likely to have a protracted course of disease that in addition was more severe, often required hospitalization and led to excess mortality than other antibiotic-sensitive *S*. Typhimurium or *S*. Enteritidis infections (Helms et al., 2002; Varma et al., 2005). Additionally, it was also suggested that due to their antimicrobial resistance, such infections may be more difficult to treat. In contrast, other studies in the United Kingdom have indicated that MDR *S.* Typhimurum DT 104 was no more or no less invasive than other Typhimurium phage types (Threlfall et al., 1998). Furthermore, the possibility that *S.* Typhimurium DT 104 exhibits enhanced virulence could not be confirmed by later work using DNA microarray analysis (Litrup et al., 2010). It is possible that 'epidemic' strains of *Salmonella* may 'lose' virulence or antimicrobial resistance over time due to mutations that adversely affect the 'fitness' of the strains in the given environment (Andersson and Hughes, 2010).

The occurrence of antimicrobial resistance in *Salmonella* in poultry production in the EU and food-borne antimicrobial resistance as a biological hazard was reviewed in previous EFSA Opinions (EFSA, 2009, 2010d). Recent data indicate that the occurrence of resistance against several antimicrobials in *S.* Typhimurium and *S.* Enteritidis isolates from humans resembles the occurrence of antimicrobial resistance reported for these serovars in poultry (*Gallus gallus*) (ECDC et al., 2009).

Increasingly, microbiologists and clinicians are faced with bacteria that produce enzymes able to hydrolyse third generation cephalosporins, such as cefotaxime and ceftazidime, which are used widely in empirical and specific regimens for human bacterial sepsis. These so-called extended-spectrum beta-lactamases (ESBLs) and AmpC beta-lactamases are usually encoded by genes present on transferable plasmids, which often encode resistance to several other antibiotic classes, such as aminoglycosides, trimethoprim and fluoroquinolones (examples of *S*. Paratyphi B variant Java in the Netherlands (van Asselt et al., 2009; van Pelt et al., 2003). An example of an "epidemic MDR strain with AmpC, CMY-2 based cephalosporin resistance" is *S.* Newport in the USA and Canada (Gupta et al., 2003; Weir et al., 2004). There are other serovars with MDR, and these are found at unusually high prevalence in the broiler industry of certain Member States like, for example, *S*. Infantis in Hungary, *S*. Mbandaka and *S*. Paratyphi B variant Java in Germany and in The Netherlands, *S*. Kentucky in Ireland (EFSA, 2007d). These aspects indicate that, at least in some MSs, such serovars should be considered to be of special concern for human health. Reviews of the last 5 years data on *Salmonella* isolates from poultry and poultry products carrying third generation cephalosporin beta-lactam resistance genes are available (Batchelor et al., 2005; Cloeckaert et al., 2007; Li et al., 2007). Evidence for plasmid-regulated quinolone resistance in *S*. Infantis of broiler origin is also available (Kehrenberg et al., 2006), together with evidence for plasmid mediated quinolone resistances in other *Salmonella* serovars in the United Kingdom mainly related to travel and imported food (Hopkins et al., 2007) are also available.

As yet, with the exception of the clonal expansion of *S.* Paratyphi B variant Java exhibiting resistance to extended spectrum beta-lactamases in poultry flocks in the Netherlands (MARAN, 2009), such resistance has remained at low incidence in food animal isolates in most MSs. In human, infections with *Salmonella* strains exhibiting resistance to ESBLs have been reported, but for the most part these have been confined to patients who have acquired their infections outside the EU.

In contrast to *S*. Typhimurium, MDR in *S*. Enteritidis in most EU MSs is relatively rare, as evidenced by retrospective studies in Italy and in the UK, and in almost all MSs (EFSA, 2010b) Resistance to quinolone antibiotics increased in human and poultry isolates of both *S*. Typhimurium and *S*. Enteritidis in MSs in the early 2000s (Fisher, 2004), and such increases have also been observed in human isolates of these two serovars from 2005 to 2006 (EFSA, 2010b). The serovars in which resistance to quinolone antibiotics is most common are *S*. Enteritidis, S. Infantis, S. Virchow and *S*. Hadar. In such serovars resistance to this group of antimicrobials has resulted from single point mutations in the DNA gyrase genes. Occasionally acquired resistance to quinolones related to plasmid- mediated Qnr genes has been reported in some serovars (Cattoir et al., 2007; Hopkins et al., 2008; Hopkins et al., 2007; Kehrenberg et al., 2006), but the present significance of such plasmid-mediated *qnr* genes is debatable, as they do not significantly increase the Minimum Inhibitory Concentration of carrying strains to fluoroquinolones.

Data concerning the antimicrobial resistance of the less common serovars are sparse. Even when considering *S*. Enteritidis and *S*. Typhimurium, it is difficult to make any inference because of the lack of data homogeneity and data stratification among the monitoring programmes of the different MSs. In addition, the situation of antimicrobial resistance according to the specific category of animals (e.g. layers and broilers are often reported together as poultry) is a level of detail that the MSs seldom report.

EFSA (EFSA, 2007b) has therefore proposed a harmonised monitoring scheme of antimicrobial resistance in *Salmonella* in *Gallus gallus*. Also, it proposed a common set of antimicrobials to be included using common epidemiological cut-off values to determine the susceptibility of *Salmonella* and *Campylobacter*. Based on the Decision of Commission (2007/407/EC), results of the harmonised monitoring should be reported in accordance with Article 9 of Directive 2003/99/EC, in the annual report on trends and sources of zoonoses, zoonotic agents and antimicrobial resistance. Although it seems that comparability of the data could be improved in the future, the first two reports of EFSA on the results of monitoring of antimicrobial resistance of zoonotic agents in the EU has provided a useful insight into antimicrobial resistance of *Salmonella* in broilers (EFSA, 2010b).

Antimicrobial resistance of non-typhoidal *Salmonella* has been increasing over the last two decades, both in terms of occurrence of specific resistances and prevalence of MDR strains and increasing MICs, although the level and extent of resistance vary according to different regions and in different serovars. Several mobile genetic elements (i.e. plasmids, transposons, genomic islands) play an important role in the horizontal transfer of antimicrobial resistance, thereby helping resistance determinants to spread among bacteria due to horizontal gene transfer. A further important method of spread is by the transmission through the food chain of strains with chromosomal resistance, such as seen with strains of *S.* Enteritidis with chromosomal resistance to quinolone antibiotics. In those instances the use of that type of antibiotics in poultry has been important in promoting the spread of such strains (Malorny et al., 2003).

International organizations (i.e. WHO, OIE, EMEA), as well as EFSA, survey the problem periodically, and provide analysis of the data. Recently, the Codex Alimentarius Commission has established in 2006 an *ad hoc* Intergovernmental Task Force on Antimicrobial Resistance[7]. The aim of this Task Force is to develop science based guidance to assess the risk of human health associated with the presence of antimicrobial resistant microorganisms and resistance determinants. According to the Directive 2003/99/EC, resistance to antimicrobial agents should be monitored in zoonotic bacteria

[7] http://www.who.int/foodborne_disease/resistance/codextf/en/index.html

including *Salmonella* and in commensal (indicator) bacteria. EU member states presently generate and report these data in different ways. Examples of integrated reporting systems include DANMAP in Denmark[8] and NETHMAP in the Netherlands[9].

The serovars from isolates in the UK that most frequently show MDR are *S*. Typhimurium, *S*. Paratyphi-B (d-tartrate-positive *Salmonella enterica* serovar Paratyphi B var Java: from imported poultry meat and infections), *S*. Hadar, *S*. Virchow, *S*. Heidelberg, MDR seems to be most characteristic of *S*. Typhimurium (EFSA, 2009). The MDR region of *S*. Typhimurium DT 104 resides in the 46 kb genomic island (SGI-1), which has been shown to be transmissible by P22-like phages (Cloeckaert and Schwarz, 2001) and by mobilisation(Doublet et al., 2005). Such strains are characterized by the penta-resistant (ACSSuT) phenotype (Threlfall, 2000). The ability to become resistant varies considerably between *Salmonella* serovars. *Salmonella* spp. from *Gallus gallus* includes serovars such as *S*. Typhimurium, *S*. Hadar and *S*. Virchow, which are often more resistant than *S*. Enteritidis. It is also remarkable that MDR is more than 10 times more frequent in *S*. Typhimurium than in *S*. Enteritidis, while quinolone (nalidixic acid) resistance is about twice as frequent in *S*. Enteritidis as in *S*. Typhimurium. The differences between antimicrobial properties of S. Enteritidis and S. Typhimurium have been the focus of recent attention regarding the relative roles of characteristics of the organism, opportunities for clonal expansion, the predominant food animal hosts and exposure to relevant antimicrobials (EFSA, 2010b). Among *S*. Typhimurium isolates from *Gallus gallus,* the occurrence of resistance to tetracyclines, ampicillin and sulphonamides was high and the resistance levels in the MSs that reported were 17%-34%, 17%-39% and 11%-39%, respectively, during the reporting years. Resistance levels for nalidixic acid and ciprofloxacin were 0%-22% and 0%-17%, respectively. Cefotaxime and ceftiofur resistance was not reported in *S*. Typhimurium isolates from *Gallus gallus*. The designation of resistance varies considerably according to the interpretation standards that are used; for example, cut off levels for some fluoroquinolones and cephalosporins are higher in Clinical and Laboratory Standards Institute guidance documents than in British Society for Antimicrobial Chemotherapy standards.

In conclusion, antimicrobial resistance has increased dramatically in some serovars of *Salmonella* in EU MSs over the past decade. Of particular note has been the increased occurrence of resistance to quinolone antibiotics in poultry-related serovars and phage types, such as *S*. Enteritidis of a range of phage types, and also multiple resistance in organisms such as the newly-emerging monophasic *S*. Typhimurium-like strains, which are predominantly associated with pigs. In this respect the development of resistance to antibiotics such as quinolones and fluoroquinolones, which are regarded as 'Critically-Important' by the WHO (ECDC et al., 2009) is regarded particularly undesirable.

If sufficient information becomes available to point at particular clones of special public health significance (e.g. clones with high virulence or resistance towards antimicrobials deemed critically important for empirical treatment of human infections, but not necessarily related to particular serovars), the inclusion of such clones as part of the EU-wide targets should be considered. This will, however, require that MSs are able to apply harmonised and standardised methods in order to identify these clones unambiguously.

### 4.5. Concluding remarks on *Salmonella* serovars with public health significance related to broiler meat production

- In EU, the overall reported incidence of human salmonellosis has been decreasing from 2006 to 2009, which is mainly explained by a downward trend in the number of *S*. Enteritidis infections presumably as a result of an improved surveillance and control of *S*. Enteritidis in breeding hens and laying hens in many MSs. In contrast, the absolute reported incidence of *S*. Typhimurium infections has increased indicating that one or more sources of these infections are increasing in importance.

[8] www.danmap.org/
[9] www.swab.nl/swab/swabcms.nsf/(WebFiles)/E32F6709B7DB7F2EC125744F002ACAA5/$FILE/NethMap_2008.pdf

- *S.* Enteritidis and *S.* Typhimurium are considered of paramount public health significance. Together, they account for approximately 75% of all serotyped human isolates. Other serovars constitute less than 2% on an individual basis.

- Any serovar that is not animal host-specific is considered capable of causing gastro-intestinal illness of varying severity in humans, and thus should be considered of potential public health significance. Nevertheless, temporal associations between some serovars and particular animal reservoir may occur, which impact the frequency of human illness and consequently may affect food safety decision making.

- At the EU level, the control of *S.* Enteritidis and *S.* Typhimurium should provide relatively greater public health benefit. Thus, maintaining stringent targets and controls at the EU level for *S.* Enteritidis and *S.* Typhimurium in broiler flocks is recommended.

- It is underlined, however, that targeting specific serovars very strictly may create a niche for other and possible emerging serovars and could result in a relative or absolute increase in the occurrence of such serovars or strains. New zoonotic *Salmonellae*, such as the monophasic variants of *S.* Typhimurium, may arise unpredictably and there is a need for mechanisms to identify the emergence of such strains at an early stage

- Close monitoring of the serovar distributions in humans and broilers should be strengthened to identify the emergence of serovars of public health significance.

- Targeted control of other *Salmonella* serovars in broiler flocks should be guided by the level of their occurrence in individual EU MSs.

- If sufficient information becomes available to point at particular clones of special public health significance (e.g. clones with high virulence or resistance towards antimicrobials deemed critically important for empirical treatment of human infections, but not necessarily related to particular serovars), the inclusion of such clones as part of the EU-wide targets should be considered. This will, however, require that MSs are able to apply harmonised and standardised methods in order to identify these clones unambiguously.

# 5. Estimating the public health impact of *Salmonella* in broiler meat production

## 5.1. Public health relevance of *Salmonella* in broiler meat production

Chicken meat is a well-recognised source of human salmonellosis, but its absolute or relative contribution compared to other important sources is not known for most countries or for the EU as a whole (EFSA, 2008b). In general, the relative importance is expected to vary between countries and regions depending on differences in prevalence, consumption patterns and culinary preferences, as well as farm and food production systems.

In the following, source attribution studies for human salmonellosis are presented and discussed with the focus on the role of broiler meat for human salmonellosis. *Source attribution* is defined as the partitioning of the human disease burden of one or more food-borne infections to specific sources, where the term *source* includes animal reservoirs and vehicles, e.g., foods. Methods for source attribution of food-borne diseases include microbiological approaches, epidemiological approaches, intervention studies and expert elicitations. For a thorough review of source attribution methods see (EFSA, 2008b) and (Pires et al., 2009).

### 5.1.1. Source attribution using microbial subtyping

Several models have applied microbial subtyping data in order to make inferences about the most important sources of human infection. The basic principle of these types of model is to compare the distribution of subtypes in potential sources (e.g. animals and food) with the subtype distribution in

humans. In the case of *Salmonella*, serotyping and phage-typing, sometimes combined with AMR patterns, have so far been the preferred typing methods used for this purpose.

The microbial subtyping approach has proved to be a valuable tool in focusing food safety interventions to the appropriate animal reservoir in Denmark (Korsgaard et al., 2009; Wegener et al., 2003) and the Netherlands (Van Pelt et al., 1999), and the model developed in Denmark (Hald et al., 2004) has recently been adapted to attribute human salmonellosis in other countries (Mullner et al., 2009; Pires et al., 2008).

In a study of Danish surveillance data since 1989 (Korsgard et al., 2009), the results indicate that the number of human *Salmonella* cases that can be associated with the consumption of broiler meat has been declining since the first initiative to control *Salmonella* in broiler production was implemented in 1989 and was later followed up by revisions in 1994 and 1997 (Figure 5). Over this time period, the *Salmonella* flock prevalence in broilers was reduced from around 75% in 1989 to 9% in 1996 and 1.1% in 2009.

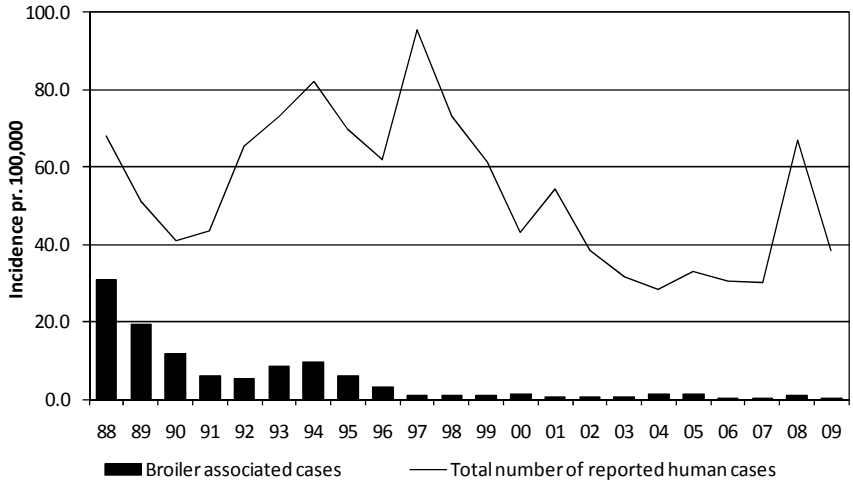



**Figure 5:** Reported number of total human salmonellosis cases per 100,000 population and estimated number of broiler associated human cases in Denmark from 1988 to 2009 (Updated from Wegener *et al.*, 2003).

Source attribution information broken down by serovars associated with *Gallus gallus* is available only in a few MSs. Results from the Netherlands and Denmark are presented in Table 4 from which it can be seen that *S.* Enteritidis is estimated to be associated with the majority of human cases from broiler meat in The Netherlands and from imported broiler meat in Denmark, whereas *S.* Enteritidis originated from domestically produced meat only constitutes a very small proportion of the broiler-associated cases in Denmark. In both countries, broiler meat does not appear to be a very important source of human *S.* Typhimurium infections, although broiler meat produced in Denmark does seem to contribute relatively more to *S.* Typhimurium infections than to *S.* Enteritidis infections.

**Table 4:** Proportion of sporadic national cases (i.e. excluding outbreak and travel related cases) and serovar distribution of human salmonellosis attributed to laying hens, broilers (*Gallus gallus*) and products hereof based on microbial subtyping between 2003 and 2009 in The Netherlands and Denmark. Raw data and calculations kindly supplied by Wilfrid Van Pelt (RIVM, Bilthoven, The Netherlands) and Tine Hald (FOOD-DTU, Soborg, Denmark).

| **Country** | **The Netherlands 2005-2009** | | | **Denmark 2005-2009** | | | |
|---|---|---|---|---|---|---|---|
| **Reservoir/vector** | **All endemic sources** | **Broilers, broiler meat** | **Layers** | **All endemic sources** | **Broilers National** | **Broiler meat, imported** | **Layers** |
| Attributable fraction (all serovars) | | 11.8% | 28.0% | | 2.2% | 7.3% | 6.4% |
| *S.* Enteritidis | 33.9% | 4.6% | 21.9% | 31.9% | 0.1% | 3.3% | 5.6% |
| *S.* Typhimurium | 35.2% | 1.8% | 2.3% | 28.0% | 0.3% | 0.4% | 0.5% |
| *S.* Hadar | - | 0.4% | 0.0% | - | 0.0% | 0.1% | 0.0% |
| *S.* Infantis | - | 0.7% | 0.2% | - | 0.2% | 0.3% | 0.0% |
| *S.* Virchow | - | 0.5% | 0.4% | - | 0.1% | 0.6% | 0.0% |
| Other serovars | - | 3.9% | 3.1% | - | 0.7% | 2.5% | 0.3% |

Under the scope of the "Source Attribution" working group supported by the European Network of Excellence Med-Vet-Net, the Danish model was adapted to include surveillance data from the United Kingdom, the Netherlands and Sweden (Figure 6) (Pires et al., 2008). The results of the study suggested that broiler meat only seems to contribute to a limited extent in three of the four participating countries and that the most important sources of human salmonellosis varied between countries, presumably reflecting different control strategies and consumption patterns. The study also showed that international travel is an important risk factor in all countries. It is emphasized that the data used in these models represents different years, so the estimates provided do not necessarily reflect the current situation. For instance in UK, a lot of effort had gone in to control *Salmonella* in broilers in recent years, so the number of cases associated with the consumption of chicken is expected to be now lower than in the period of the data used in the study. This implies that if this modelling approach is applied on a regular basis, it would also allow for the quantitative analysis of trends for the most important sources for food-borne salmonellosis over time.

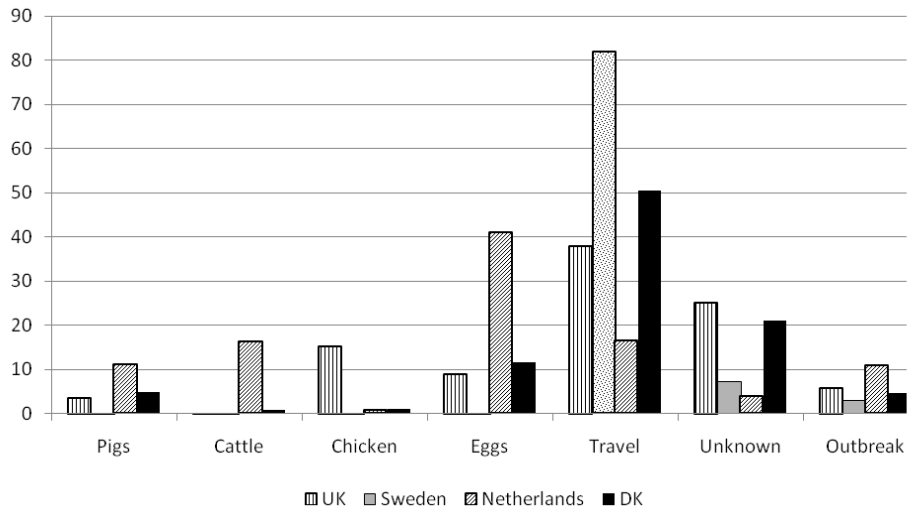



**Figure 6:** Estimated proportion (%) of Salmonella cases attributed to specific sources in the UK (data from 2003-5), Sweden (2004-6), the Netherlands (2006) and Denmark (2007) (%) (Pires et al., 2008). Percentages do not add to 100%, since some sources were excluded to enhance comparability.

### 5.1.2. Source attribution using outbreak data

Another way of trying to assess the proportion that is likely to be food-borne, and the foods implicated in causing human disease, is to use data from outbreak investigations. In contrast to the microbial subtyping approach, which primarily works at the reservoir level, outbreak data are observed at the public health endpoint. A simple descriptive analysis or summary of outbreak data is useful for attributing illnesses to foods (Adak et al., 2005; Greig and Ravel, 2009), but often the implicated food is a "complex" food containing several food items, where any of the items could be the actual source of the infection.

An alternative method for conducting an analysis of data from outbreak investigations was developed in the United States. In this method, food items are categorized into a hierarchical scheme, according to their ingredients and the developed model is able to attribute human cases from complex food to specific simple food categories (Painter, 2006).

This method has recently been adapted to attribute human salmonellosis in Europe . Based on food-borne outbreak data collected by EFSA for the reporting years 2005 and 2006, the authors estimated that the most important food sources were eggs (32%) and meat, including poultry-meat (15%), but also concluded that a large proportion of cases could not be linked to any source. Among illnesses that could be linked with a specific source, 58% of salmonellosis cases were attributed to eggs. Results revealed regional differences in the relative importance of specific sources (Figure 7).

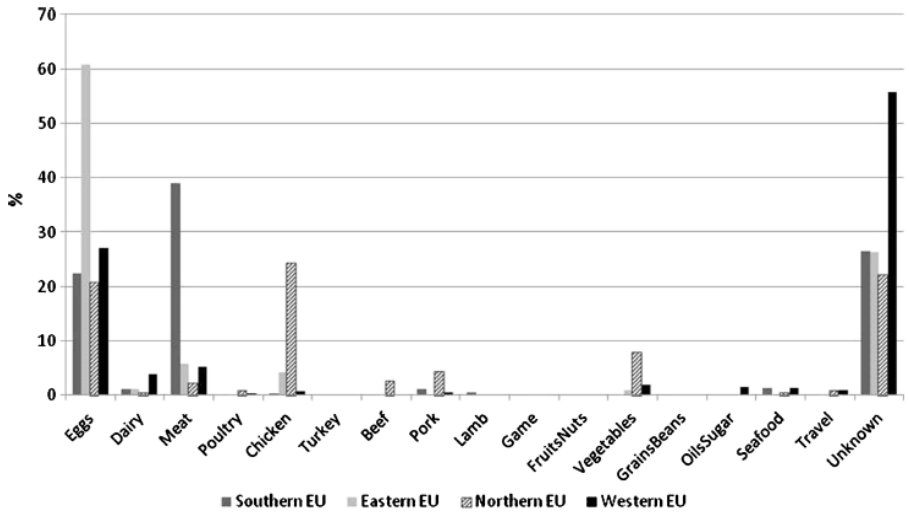



**Figure 7:** Proportion of salmonellosis-outbreak-associated cases attributed to specific sources, travel, and of unknown origin in different European regions (Pires et al., 2010).

Limitations of using of outbreak data for attribution include that the quality of evidence varies between data sources and classification schemes for the data are not consistently used. Also, large outbreaks, outbreaks associated with point sources, outbreaks that have short incubation periods, and outbreaks that cause serious illness, are more likely to be investigated. Likewise, certain food vehicles are more likely to be associated with reported outbreaks than others, which can lead to an overestimation of the proportion of human illnesses attributed to a specific food. An important factor to consider is that illnesses included in data from outbreak investigations may not be representative of all food-borne illnesses. The fraction of the burden of food-borne disease that is associated with outbreaks varies between pathogens but is typically smaller than the correspondent to sporadic disease. Consequently, the extrapolation of source attribution estimates obtained through an analysis of data from outbreaks to the overall burden of disease should be made with caution. The authors of the above study, however, concluded that the approach appeared to be useful for attributing human salmonellosis.

## 5.2. Consumption of broiler meat and products thereof in the EU

Exposure of humans to *Salmonella*-contaminated broiler meat does not only depend on the prevalence of *Salmonella*, but also on the amount consumed and preparation of the food. Differences in consumption patterns may therefore lead to differences in exposure levels between MSs. There are several sources that provide data on food consumption, but none of these specifically addresses consumption of broiler meat, but rather the total consumption of poultry meat i.e. also including meat from poultry species other than *Gallus gallus*. Appendix D includes a short review of sources with available data on human consumption of poultry meat in the EU.

Overall, it is difficult to compare the consumption data provided from the different sources investigated because data collection and reporting methods as well as reporting year differ between surveys. Figure 8 was created using the data reported through the FAOSTAT database (FAOSTAT[10], accessed 27 February 2011) as this dataset provides data for all MSs for 2007 and is assumed to be the most up to date and comparable of the three datasets presented in Appendix D. From the FAOSTAT data, it can be concluded that there are considerable differences between the EU MSs with regard to the amount of poultry meat consumed (range: 13-39 kg per capita per year). The mean consumption in all of EU was 20 kg/capita/yr in 2007 and this amount appears to have been quite stable through the last 5 years (FAOSTAT).

The annual report 2009 from the Association of Poultry Processors and Poultry Trade in the EU Countries includes specific data on broiler meat consumption for a few EU MSs (Table 1, Appendix D). In these MSs, the consumption of broiler meat constitutes between 55% in France to 82% in The Netherlands of the total amount of poultry meat consumed. It is, therefore, considered likely that well over half of the poultry meat consumed in EU is broiler meat. In the MSs with figures for broiler meat consumption, there seems to be a slightly increasing trend in the period from 2003 to 2008 (AVEC, 2009).

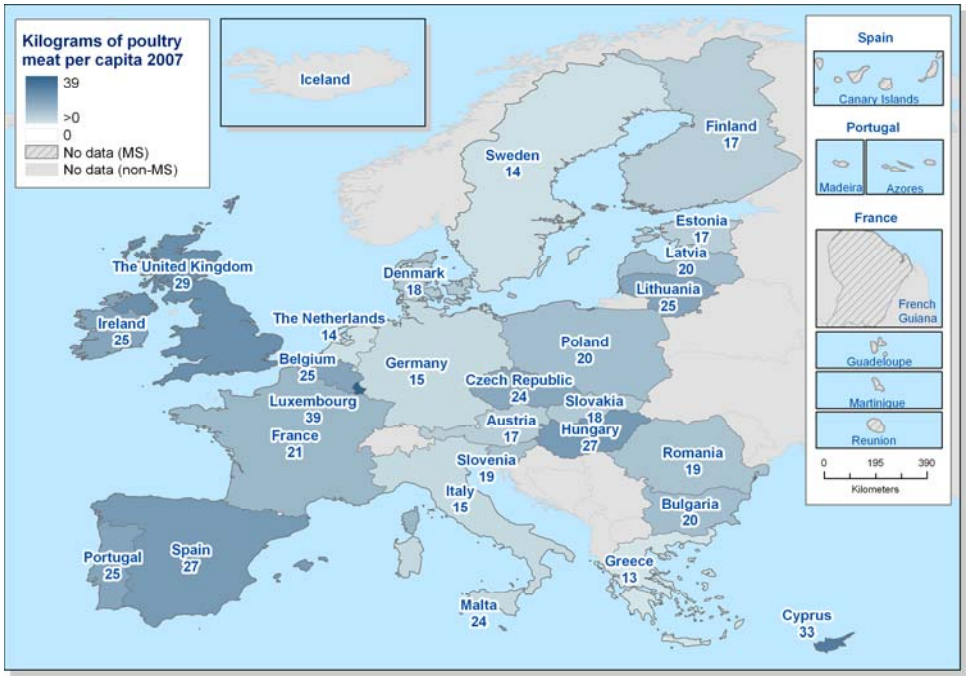



**Figure 8:** Consumption of poultry meat in EU Member States (FAOSTAT, accessed 27/02/11). Data used are presented in Table 1 in Appendix D. Figure kindly provided by the EFSA Unit on Zoonoses data collection.

[10] http://faostat.fao.org/default.aspx

Food consumption data available from the EFSA Food Consumption Database was also reviewed and taken into account. However, due to the between MSs variability regarding survey years, level of detail and survey systems employed, the use of FAOSTAT data was considered to be more appropriate in this case.

## 6. Risk assessment methods and data

In order to answer the questions put forward by the Commission in the Terms of References, the chosen modelling approach would have to be able to:

1. Estimate the relative (or absolute) contribution of broiler meat to the burden of human salmonellosis in EU, and

2. distinguish between the recognised, but uncertain, differences between the various serovars in their ability to cause human disease.

In addition, the modelling approach needs to include the prevalence observed during monitoring in the MSs as an explanatory variable. The human health impact as a consequence of a change in this value should be the main outcome of the model.

For this purpose, the WG identified two possible modelling approaches, the "traditional" farm-to-consumption risk assessment modelling and the Bayesian microbial subtyping approach. The principles, advantages and limitations of both are discussed below.

### 6.1. Farm to consumption modelling

Risk assessment, along with risk management and risk communication, is one of the components of risk analysis, which can be defined as an overall strategy for addressing risk. This framework was initially defined by FAO, WHO and the Codex Alimentarius Commission in 1995 (FAO/WHO, 1995).The importance of an overlap between these three elements (risk assessment, risk management and risk communication) is well recognised, but some functional separation is also necessary. In relation to risk assessment, such separation ensures that issues are addressed in a transparent manner with a scientific basis.

A risk assessment process is a means of providing an estimate of the probability and severity of illness attributable to a particular pathogen-food combination. Conceptually, a typical farm-to-consumption quantitative microbiological risk assessment (QMRA) starts from the dynamics of the hazard in the food chain and uses predictive models to estimate the outcomes in terms of public health. The models describe the changes in prevalence and pathogens numbers through the production chain. Data on prevalence (e.g. at farm, slaughter and/or retail level) and microbial loads, as well as the effect of the different production processes on the prevalence and load, therefore, serve as essential inputs to a QMRA model. The exposure assessment consists of a chain of conditional probability distributions, where the outcome is the probability distribution of the prevalence and microbial load of a given pathogens at the time of serving/consumption. These estimates are then linked with the dose-response model to estimate the probability of illness. From this it follows that the results of a stochastic QMRA are presented as probability distributions and may include probability distribution of prevalences, microbial loads and number of human illnesses, but the level of detail in which each step is addressed and the definition of the exact outcomes will depend on the scope of the risk assessment as well as the availability of data. The scope should be defined clearly by the risk manager and discussed thoroughly with the risk assessor.

The QMRA approach has a high resolution in the food chain and can provide valuable information on the complex dynamics of pathogens during food processing. It is considered a particular useful risk management tool for evaluating the relative impact of specific interventions and predicting the effect of potential intervention. It is also regarded as the only approach that in principle allows the high level of detail needed for e.g. estimating the proportion of cases attributable to a specific food commodity,

like for example minced meat (EFSA, 2008b). However, comprehensive and detailed AMRA exercises are time consuming and resource intensive. Practical and financial constraints such as time, expertise, data availability and additional resources are considered major limitations. Particular insufficient data including limited availability of dose-response information often makes it necessary to apply a number of more or less plausible assumptions. The approach is therefore also recognized as being less useful for predicting actual public health outcomes.

Several risk assessments relating to *Salmonella* in broilers and chicken meat have been conducted and published. Appendix F gives an overview with a short summary of the objectives and major findings of risk assessments and other relevant studies conducted within this area.

### 6.2. Bayesian microbial subtyping modelling approach

The microbial subtyping approach involves characterisation of isolates of the pathogen by phenotypic and/or genotypic subtyping methods. The principle is to compare the distribution of subtypes in potential sources (e.g. animals and food) with the subtype distribution in humans and it is enabled by the identification of strong associations between some of the dominant subtypes and a specific food-animal reservoir, providing a heterogeneous distribution of subtypes among the sources. Subtypes exclusively or almost exclusively isolated from one source are regarded as indicators for the human health impact of that particular source, assuming that all human infections with these subtypes originate only from that source. Human infections caused by subtypes found in several reservoirs are then distributed relative to the prevalence of the indicator types.

The principle of comparing the distribution of subtypes found in animal and food sources with those found in humans to make inferences about the most important sources of human disease has been applied by several research groups (Sarwari et al., 2001; Van Pelt, 1999). A Bayesian model developed to attribute human salmonellosis in Denmark (Hald et al., 2004) has regularly been improved to include data on antimicrobial susceptibility (Hald et al., 2007) as well as data from multiple years (Pires and Hald, 2010).

The model attributes domestically acquired laboratory-confirmed human infections caused by different subtypes (e.g. serovars, phage types, antimicrobial resistance profiles) as a function of the prevalence of these subtypes in animal and food sources and the amount of each food source consumed. However, the number of people being infected by a particular subtype in a particular food source supposedly depends on additional factors related to the subtype and food source in question. Therefore, a multi-parameter prior, which accounts for the presumed but undefined differences between subtypes and food sources with respect to cause human infections was introduced.

The bacteria-dependent factor {qi} can be interpreted as combining survivability, virulence, and pathogenicity of the pathogen to estimate the ability of that subtype to cause disease, whereas the food source dependent factor $\{a_j\}$ estimate the ability to act as a (protective) vehicle for food-borne infections as well as the sensitivity of monitoring programme used to obtain the data included in the model. It is, however, emphasised that the estimated values of the bacteria- and food-source-dependent factors are simply multiplication factors (comparable to regression coefficients in regression analyses) that helped us arrive at the most likely solution given the observed data. Their relative size can provide an idea about the differences between *Salmonella* types and food types with respect to causing human infections, but estimates based on the results of a single model should be interpreted with care. However, by applying the model on a regular basis as new data becomes available, it may be possible to monitor the main sources and dynamics in the occurrence of human salmonellosis and to improve the estimation of the model parameters including the bacteria- and food-dependent factors.

The basic equation used to estimate the number of human cases per source and type is defined as follows:

$$\lambda_{ij} = p_{ij} * M_j * a_j * q_i \qquad \text{(Equation 1)},$$

where $\lambda_{ij}$ is the expected number of cases per subtype *i* and source *j*, $p_{ij}$ is the prevalence of subtype *i* in source *j*, $M_j$ is the amount of source available for consumption in the country, $a_j$ is the food source dependent factor for source *j*, and $q_i$ is the bacteria dependent factor for type *i*. To avoid problems related to identifiability (i.e. overparameterisation) of the model described in Eq. 1, the number of estimated parameters needs to be reduced. The pooling of some subtypes or food sources into groups with similar characteristics is one way of addressing this problem, but other approaches may(Straver et al., 2007) also be useful. Depending on the available data, the model can be extended to include other dimensions such as time period (e.g. year) and country, which can also increase the robustness of the model and consequently improve the parameter estimation for instance by assuming that the q-values remain unchanged over at least shorter time periods (Pires & Hald, 2010) or are independent on country.

The model calculates the expected number of cases per subtype $\{\boldsymbol{\lambda_i}\}$ according to the above equation. From this $\lambda_i$, a back-calculation is made by adding the number of travel- and outbreak-related cases with known phage type in order to get the expected number of reported cases. The observed data (i.e. the reported number of cases per subtypes) is then linked with the prior distribution by assuming that the number of cases per subtype is Poisson distributed (the likelihood function) with a parameter value equal to the expected number of cases. This results in posterior estimates for the unknown parameters $q_i$ and $a_j$ and consequently for the number of cases per subtype and source $\{\boldsymbol{\lambda_{ij}}\}$, which can then be summarised over subtypes to get to the number of cases per source $\{\boldsymbol{\lambda_j}\}$.

The microbial subtyping approach requires a collection of temporally and spatially related isolates from various sources and humans, and is consequently facilitated by an integrated food-borne disease surveillance programme focused on the collection of *Salmonella* isolates from the major food animal reservoirs and from humans (Pires et al., 2009). The data quality and availability are considered the biggest limitation of this approach.

A strong advantage of the microbial subtyping approach is that it allows for the identification of the most important reservoirs of *Salmonella*, assisting risk managers to prioritize interventions and focus control strategies at the animal production level. Particularly, if repeated on a regular basis, the approach is regarded as a powerful tool to monitor the progress of *Salmonella* control and follow the trends in the sources of human salmonellosis (Hald et al., 2004; Pires et al., 2009).

The results of this type of model can also provide estimates for the effect on the number of human cases originating from a particular reservoir (e.g. broilers), if the observed prevalence in that reservoir is changed for instance following the implementation of a control program. Given the nature of the model, it will also be able to provide estimates on the expected change in human cases for specific subtypes, e.g. specific serovars of *Salmonella*.

However, in contrast to a "traditional" farm-to-consumption risk assessment model, the model does not give detailed insight into transmission routes and cannot provide estimates for the expected changes in human infections by the introduction of specific intervention strategies.

### 6.3. Model choice

As described in the introduction of this Scientific Opinion, EFSA launched an open call for tender for the provision of a modelling work in order to support the estimation of the quantitative aspects inherent to the request made by the European Commission.

Out of this open call and following EFSA outsourcing procedures, a contractor who met the technical and administrative criteria of the call was selected. The model employed by this contractor, which is thoroughly described in the report of the contractor (VOSE, 2011) must be read together with this Scientific Opinion. The model is based on the Bayesian approach using microbial subtyping data described above, but with MSs added to the model as a third dimension. This type of modelling

approach would have the following advantages when compared to the farm-to-consumption modelling approach:

- The subtyping approach is serovar specific meaning that it can handle the influence of individual serovars and the assumed difference between them with regard to causing human illness. This is a necessity in order to answer the ToRs;

- In contrast to the farm-to-consumption approach, the subtyping approach does not apply a dose-response model. Dose-response model involves a lot of uncertainty and only very sparse data is available regarding serovar-specific dose-response models;

- The data available for the project were more appropriate for the subtyping approach than for the farm-to-consumption approach, which requires detailed information on the growth, reduction and cross-contamination of *Salmonella* during the broiler production chain. Such detailed data is often insufficient with many gaps and data collection is time consuming and would probably not have been possible within the time frame given this Scientific Opinion. Finally, the subtyping approach is considered sufficient to answer the ToRs, since estimation of the effect of specific interventions are not required;

Detailed information on the methodology, mathematical principles, assumptions, data used, uncertainties and results of the model can be found in the full report provided by the contractor (VOSE, 2011). However, it is described in the body of this opinion the steps followed for selecting the data employed in the building of the 'Broiler-Target *Salmonella* Attribution Model' (BT-SAM model). The BT-SAM model serves as the initial frame for estimating the source attribution proportions. Data are considered the key element that would influence the results in quantitative modelling. Thus, the decision-making process for data use (in particular for occurrence of *Salmonella* in animal sources and its serovar distribution) largely involved the opinion of the Experts of the *ad hoc* working group elaborating the draft Scientific Opinion.

### 6.4. Data choice

The following are the key types of data employed for building the BT-SAM model:

i. Reported cases of human salmonellosis in the EU by MS and the related serovar distribution for both sporadic cases and outbreak data (TESSy[11] data kindly supplied by ECDC[12] and outbreak data from the EFSA Community Summary Reports kindly supplied by the EFSA Unit on Zoonoses data collection) covering the period 2007 to 2009. Human data supplied by ECDC in the format needed for the modelling exercise (e.g. detailed serovar information, confirmation status, origin) may have differences regarding the total number of cases when compared to the data published by ECDC and EFSA in the EU Community Summary Reports (EFSA and ECDC, 2009, 2010 and 2011). The latter publications have to be considered as the reference for the total true number of reported cases by the MSs.

   To take account for differences in underreporting between MSs, the model uses an underreporting factor calculated following the methodology as described in Appendix A. The underreporting factors were calculated based on Swedish travellers data kindly provided by the Swedish Institute for Communicable Disease Control (Smittskyddsinstitutet, SMI, Solna, Sweden, epidemiologist Sofie Ivarsson). Appendix A shows detailed calculations.

ii. Data from EUROSTAT on production, import and export data of different animal-related foodstuffs were used to calculate an approximation for the consumption of the different types

[11] ECDC, TESSy Release 1 (06/07/2010) and 2 (28/10/2010 and updated on 05/05/2011).
[12] ECDC has no responsibility for the results and conclusions when disseminating the results of the work employing TESSy data supplied by ECDC.

of food with different origin for each MS. As described by VOSE (2011) the amount available for consumption was estimated by [production-export+import] for each MS.

iii. Occurrence and serovar distribution of *Salmonella* in food-producing animal populations in the EU as reported through the Baseline Surveys and the Community Summary Reports.

For the first two types of data (human salmonellosis and amount of food available for human consumption) a detailed description on the sources and the data used in the model is presented in the contractors report (VOSE, 2011). The data employed were those that were available and best fit for purpose.

The Expert WG played a more active and fundamental role for deciding data choice regarding occurrence of *Salmonella* in animals and related serovar distribution. Depending on the different animal and/or animal products, data considered were as presented in Table 5.

**Table 5:** Data sources on occurrence of *Salmonella* and distribution of serovars in animals and related foods in the EU considered for the BT-SAM model.

| Animal population of interest | Data available and year(s) of data collection |
|---|---|
| Broilers | Harmonised statutory monitoring, 2009.<br>Non-harmonised statutory monitoring, 2007-2008.<br>EU-wide[1] Baseline survey in broiler carcasses, 2008.<br>EU-wide[1] Baseline survey in broiler flocks, 2005-2006. |
| Laying hens | Harmonised statutory monitoring, 2008 and 2009.<br>Non-harmonised statutory monitoring, 2007.<br>EU-wide[1] Baseline survey in holdings of laying hens, 2004-2005. |
| Slaughter Pigs | Non-harmonised EU statutory monitoring, 2007-2009.<br>EU-wide[1] Baseline survey in slaughter pigs, 2006-2007. |
| Fattening turkeys | Non-harmonised EU statutory monitoring, 2007-2009.<br>EU-wide[1] Baseline survey in turkey flocks, 2006-2007. |
| Cattle | Non-harmonised EU statutory monitoring, 2007-2009. |

[1]Some MSs did not participate in particular baseline surveys. Further information can be found in the report provided by the contractor (VOSE, 2011) as well as in the EFSA reports presenting the results of each of the baseline surveys.

The three main principles driving the selection of data were:

- Existence of a harmonised statutory monitoring among EU MSs and the duration (number of years) of this harmonised monitoring in place;
- Level of serovar detail contained in the data;
- Relevant baseline surveys carried out in recent years.

The decision tree which reflects the criteria employed for selecting the data for the BT-SAM model is presented in Figure 9.

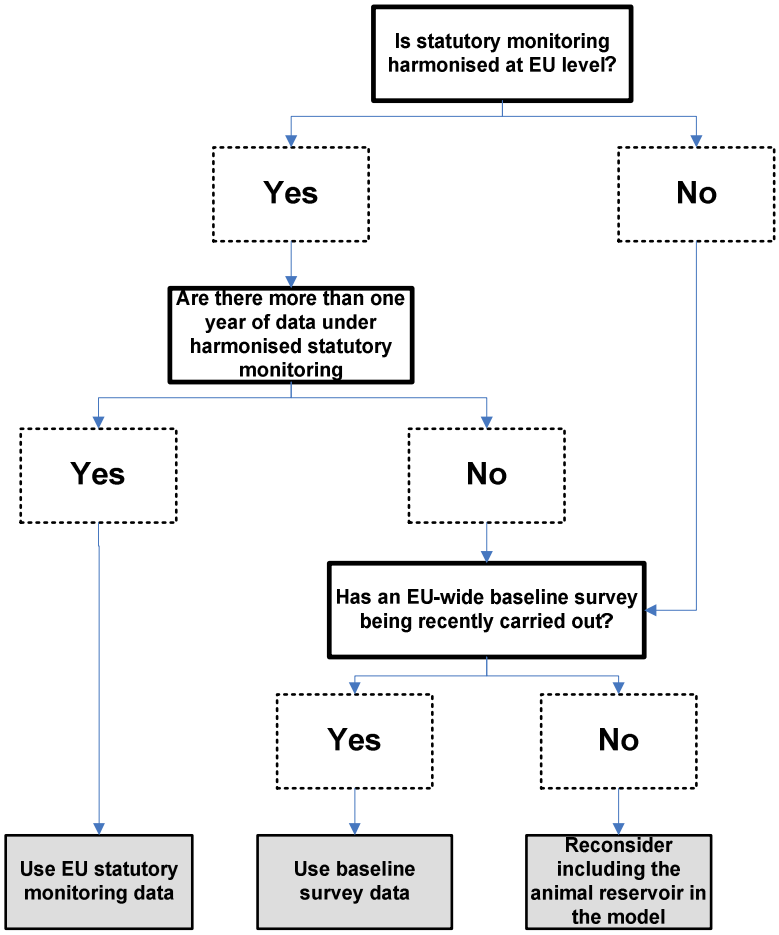



**Figure 9:** Decision tree describing the criteria for selecting data for the BT-SAM model.

Based on the criteria described in Figure 9, the following specific data choices were made (as also presented in VOSE, 2011):

- ***Broilers.***
  As the ToRs refer to targets at the flock level, the decision made was to apply the flock prevalences as obtained through the EU Baseline survey in broiler flocks conducted in 2005-06. However, since the chosen model is based on the distribution of serovars, the decision made was to apply the serovar distribution from the EU Baseline survey in broiler carcasses in 2008 in order to use as recent data as possible, where detailed information on the serovar distribution was available for the majority of MSs. By doing this, it is assumed that the serovar distribution found at the carcass level is a reflection of what the consumers are exposed to from the broiler reservoir.

- ***Laying hens.***
  The decision was to apply the EU statutory harmonised monitoring data as reported by the EU MSs in the EFSA Community Summary Report for 2008. These data are the most valid and recent data available, and should be regarded comparable between MSs.

- ***Slaughter pigs.***
  There is not at this time any harmonised monitoring going on at the EU level and only a few MSs performs bacteriological monitoring of pig herds and/or pork products. It was therefore decided to apply the EU Baseline survey data on *Salmonella* in fattening pigs conducted in 2006-2007.

- ***Turkeys.***
  As for slaughter pigs, no harmonised monitoring is taken place in EU. Data from the baseline survey on *Salmonella* in fattening turkeys conducted in 2006-2007 were, therefore, used.

- ***Cattle.***
  Only sparse data on *Salmonella* occurrence including distributions of serovars in cattle herds, beef products or dairy products are available from MSs according to the Community Summary Reports. For this reason, data from the cattle reservoir were not included in the model. The consequence of the omission of the cattle reservoir may have on the model results is discussed later.

- ***Other sources of Salmonella.***
  *Salmonella spp.* has been isolated in other animal species throughout the EU under different sampling schemes (EFSA and ECDC, 2011). These animal species include both food production animals (e.g. poultry species other than chickens and turkeys, sheep, goats and solipeds) and pets (e.g. cats, dogs, reptiles). However, only sparse data is available from MSs on its occurrence and on the serovars encountered. The consequence of the omission of these other reservoirs may have on the model results is discussed later.

The above datasets chosen for the model are assessed as currently being those providing the best comparability between MSs due to harmonized baseline studies as well as monitoring program thereby ensuring the most accurate and robust model. The model included data from 22 MSs, four animal-food sources (broilers, laying hens, pigs and turkeys) and 23 individual serovars. The 23 serovars were selected based on their presence and importance in humans and in the main animal reservoirs. This model is referred to as the BT-SAM model.The results of the BT-SAM model are then compared with the results of the model ran under different scenarios as described in the following section.

## 7. Risk assessment results

In order to provide source attribution estimates that would serve for underlining the answer to the ToRs made by the Commission, the results of the BT-SAM model were compared with the results retrieved by employing data under the following scenarios:

- **Scenario 1.** The actual prevalence of all *Salmonella* serovars as reported by the MSs in 2009. It has to be further specified that the prevalence of *S*. Enteritidis and *S*. Typhimurium were used as reported in 2009 while the distribution of the other serovars took the same ratio as they had in the EFSA baseline survey of *Salmonella* in broiler carcasses but normalised to give the total prevalence for all serovars as reported in 2009. This is because of the lack of serovar detail in the 2009 EU monitoring data reported for *Salmonella* in broiler flocks. For four MSs, the serovar distribution for other serovars from the baseline survey could not be employed due to an observed prevalence of 0% in the baseline survey. In these cases, the serovar distributions reported for 2009 were applied.

- **Scenario 2.** The actual prevalence of *S*. Enteritidis and *S*. Typhimurium in broiler flocks as reported by the MSs in 2009.

- **Scenario 3.** The combined prevalence of *S*. Enteritidis and *S*. Typhimurium = 1% (or less).

- **Scenario 4.** The prevalence of *S*. Enteritidis = 1% and *S*. Typhimurium = 0%.

- **Scenario 5.** The prevalence of *S*. Enteritidis = 0% and *S*. Typhimurium = 1%.

- **Scenario 6.** The overall prevalence i.e. of all serovars = 1% (or less).

- **Scenario 7.** The prevalence of the top-5 serovars in humans in 2009 = 1% or less (*S*. Enteritidis, *S*. Typhimurium, *S*. Infantis, *S*. Virchow and *S*. Newport).

If the prevalences in the BT-SAM model were already at or below the scenario prevalences, the baseline prevalences were kept with the exception of scenario 1 and 2, where the prevalences reported for 2009 were applied. For scenarios 2, 3, 4 and 5, the prevalences of serovars other than *S*. Enteritidis and *S*. Typhimurium were kept as in the BT-SAM model because not all MSs reported serovar-specific prevalences for these other serovars in 2009.

It was attempted to consider monophasic *S*. Typhimurium like strains as a separate subtype. However, the way this strain is reported by the MSs varies as documented by a previous EFSA Opinion (EFSA, 2010g). Thus, some MSs may report it as *S*. Typhimirium, while other MSs report different variations of the antigenic formula. This variability did not allow for their consideration as a separate group.

## 7.1. Estimates of the public health impact of different *Salmonella* flock prevalence values in broiler production

### 7.1.1. Results employing the 'Broiler-Target *Salmonella* Attribution Model' (BT-SAM)

The model included the following MSs: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Portugal, Slovakia, Slovenia, Spain, Sweden, The Netherlands and the United Kingdom.

The following MSs were excluded from the analysis because of lack of sufficient data: Bulgaria, Malta, Poland, Portugal and Romania.

The estimated changes to human incidence rates therefore only apply to the MSs included in the model.

Key results of the BT-SAM model are presented in Figure 10 and Table 6. Further detailed results can be found in the report done by the contractor (VOSE, 2011), including MS-specific source attribution estimates.

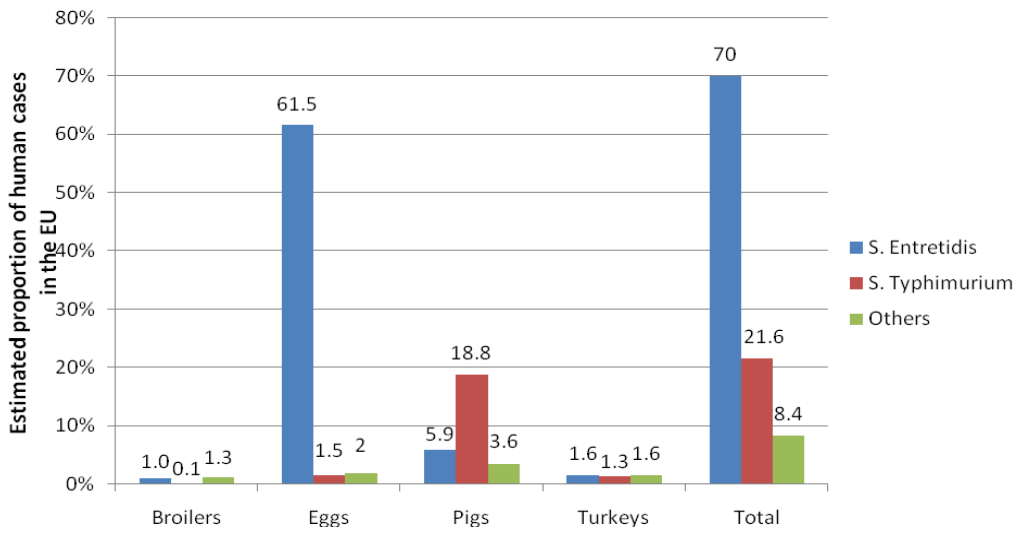



**Figure 10:** Proportion of human cases of salmonellosis (for the period 2007 to 2009) by food type and serovar (*S*. Enteritidis, *S*. Typhimurium and others) as estimated by the BT-SAM model (VOSE, 2011).

**Table 6:** Estimated total number of broiler-associated human salmonellosis cases by the 23 serovars considered in the EU MSs included in the analysis.

| ***Salmonella* serovar** | **Mean number of cases** | **% of total** |
|---|---|---|
| Enteritidis | 87,513 | 42.2% |
| Infantis | 47,665[1] | 23.0% |
| Hadar | 10,094 | 4.8% |
| Typhimurium | 9,649 | 4.7% |
| Kentucky | 9,097 | 4.4% |
| Virchow | 8,843 | 4.3% |
| Java | 7,408 | 3.6% |
| Brandenburg | 6,586 | 3.2% |
| Montevideo | 5,037 | 2.4% |
| Agona | 3,820 | 1.8% |
| Livingstone | 2,961 | 1.4% |
| Mbandaka | 2,084 | 1.0% |
| Derby | 1,350 | 0.7% |
| Anatum | 1,271 | 0.6% |
| Kottbus | 1,236 | 0.6% |
| Braenderup | 893 | 0.4% |
| Newport | 675 | 0.3% |
| Bredeney | 607 | 0.3% |
| London | 206 | 0.1% |
| Saintpaul | 156 | 0.1% |
| Heidelberg | 99 | 0.05% |
| Bovismorbificans | 0 | 0.0% |
| Rissen | 0 | 0.0% |
| **Total** | 207,250 | 100.0% |

[1]A single MS contributes with almost 90% to these total cases.

The results indicate that:

- Around 2.4% (95% CI: 1.8-3.4) of all human salmonellosis cases (i.e. estimated true number of cases when accounting for underreporting) in the EU were attributed to broilers. This is estimated to correspond to around 207,000 human cases in a three year period.

- For the broiler-associated cases, around half of the broiler-associated human salmonellosis cases were caused by serovars other than the currently regulated serovars *S.* Enteritidis and *S.* Typhimurium.

- *S.* Enteritidis, and *S.* Infantis constituted 42% and 23% of all broiler-associated cases respectively. *S.* Hadar, *S.* Typhimurium, *S.* Kentucky and *S.* Virchow constituted individually between 4% and 5% of all broiler-associated cases. It should be noted that around 90% of the *S.* Infantis broiler-associated cases originated from a single MS. Other serovars constituted less than 4% on an individual basis.

- For the other *Salmonella* sources included, the model estimated that around 65% (95% CI: 63-67), 28% (95% CI: 27-30) and 4.5% (95% CI: 4-5) of the estimated number of human salmonellosis cases could be attributed to laying hens (eggs), pigs and turkeys, respectively.

- The majority of the *S.* Enteritidis infections are related to the laying hen reservoir (i.e. consumption of eggs), whereas *S.* Typhimurium infections originate primarily from the pig reservoir.

- However, some *Salmonella* reservoirs (e.g. cattle) were not included in the model due to lack of data. It is therefore likely that the contribution of the human salmonellosis cases allocated to the animal reservoirs included in the model have been overestimated, in particular in the case of *S.* Typhimurium.

### 7.1.2. Results of the scenario analyses

Tables 7 and 8 provide the overall summary statistics for each output and scenario. The first three statistics are percentiles (2.5, 50, 97.5) representing the low, middle and high values across the range estimated by the model. The mean represents the average, or 'centre of gravity', of the uncertainty distribution (VOSE, 2011).

**Table 7:** Estimated reduction (in %) of the total number of human cases of salmonellosis as compared to the BT-SAM model.

| *Scenario* | *2.5%* | *50%* | *97.5%* | *Mean* |
|---|---|---|---|---|
| 1 | 1.34% | 1.58% | 2.25% | 1.63% |
| 2 | 0.40% | 0.58% | 1.19% | 0.63% |
| 3 | 0.39% | 0.56% | 1.13% | 0.61% |
| 4 | 0.41% | 0.57% | 1.13% | 0.62% |
| 5 | 0.83% | 1.02% | 1.62% | 1.07% |
| 6 | 1.91% | 2.15% | 2.82% | 2.21% |
| 7 | 1.22% | 1.42% | 2.02% | 1.47% |

**Table 8:** Estimated reduction (in %) of broiler-associated human cases of salmonellosis as compared to the BT-SAM model.

| *Scenario* | **2.5%** | **50%** | **97.5%** | **Mean** |
|---|---|---|---|---|
| 1 | 62.15% | 68.96% | 75.38% | 68.99% |
| 2 | 18.46% | 25.15% | 39.67% | 26.29% |
| 3 | 18.90% | 24.27% | 37.72% | 25.40% |
| 4 | 19.59% | 24.72% | 37.60% | 25.80% |
| 5 | 40.10% | 44.35% | 54.17% | 45.12% |
| 6 | 92.90% | 93.40% | 94.12% | 93.43% |
| 7 | 58.75% | 61.87% | 67.43% | 62.22% |

Detailed tables showing the MS-specific proportions of these reductions in the mean estimates of salmonellosis rates attributed to each serovar (*S.* Enteritidis, *S.* Typhimurium and others) and food per EU Member State presented in anonymous manner) is presented in the report by the contractor (VOSE, 2011).

The scenario analyses suggest that Scenario 6, i.e. reducing the prevalence of all serovars to 1% or less, would result in the largest reduction in human cases, whereas Scenario 3 (comparing the baseline 2006-model with the theoretical prevalence of *S.* Enteritidis and *S.* Typhimurium in broiler flocks

being 1% or less) provides the least reduction although the latter estimate is very similar to the estimated reductions presented for Scenario 2 and 4. In the same manner, the results for Scenario 1 and 7 appear similar, whereas Scenario 5 is in between these two groups. Compared to the BT-SAM model, all the scenarios result in estimated reductions rates between 0.6% (Scenario 3, mean value) to 2.2% (Scenario 6, mean value) for all salmonellosis cases and between 25.4% (scenario 3, mean value) and 93.4% (scenario 6, mean value) for all broiler-associated cases.

It is noteworthy that Scenario 1, which represents the situation in broilers as reported in 2009 and consequently is the best estimate for the current situation, provides the next largest reduction. This means that unless the situation changes to the worse in some MSs (for instance by increases in the prevalence of other *Salmonella* serovars that are currently not included in the control programme), a significant reduction in the number of broiler-associated cases has already been obtained since the baseline survey in broiler flocks was conducted in 2005 and 2006.

The following key scenarios addressing the terms of reference requested by the Commission address that:

- The scenario where the prevalence of the 23 *Salmonella* serovars considered in the BT-SAM model to be as reported by the Member States in 2009 results in an estimated reduction of 69% (95% CI: 62-76) in the number of broiler-associated human salmonellosis cases compared to the situation in 2006 (i.e. the BT-SAM model). This corresponds to an estimated absolute reduction of around 143,000 (95% CI: 128,000-157,000) human salmonellosis true cases out of the 207,000 broiler-associated cases estimated by the BT-SAM model.

- The scenario where the prevalence of *Salmonella* Enteritidis and *Salmonella* Typhimurium is as reported by the Member States in 2009 (but keeping the prevalence for the other 21 serovars as per the 2005-2006 *Salmonella* Baseline Survey in broiler flocks) results in an estimated reduction in the number of broiler-associated human salmonellosis cases of 26% (95% CI: 18-40) compared to the situation in 2006. In absolute numbers, this corresponds to an estimated reduction of 54,000 (95% CI: 37,000-83,000) human salmonellosis true cases.

- The scenario where the achievement of the current target of the EU control programme of *Salmonella* in broiler flocks would be met (i.e. the combined prevalence of *Salmonella* Enteritidis and *Salmonella* Typhimurium being 1% or less, and keeping the prevalence for the other 21 serovars as per the 2005-2006 *Salmonella* Baseline Survey in broiler flocks) results in an estimated reduction in the number of broiler-associated human salmonellosis cases of 25% (95% CI: 19-38) compared to the situation in 2006. In absolute numbers, this corresponds to an estimated reduction of 52,000 (95% CI: 39,000-79,000) human salmonellosis true cases.

- The scenario where an EU-wide target is of maximum of 1% of flocks remaining positive for all the *Salmonella* serovars considered in the model would be met results in an estimated reduction in the number of broiler-associated human salmonellosis cases of 93.4% (95% CI: 92.9-94.1) compared to the situation in 2006. In absolute numbers, this corresponds to an estimated reduction of around 193,000 (95% CI: 192,000-195,000) human salmonellosis true cases.

It has to be highlighted again that the EU statutory monitoring in the MSs is likely to have a lower sensitivity than the conducted EU-wide Baseline Surveys. For this reason, the estimated reductions in number of human salmonellosis cases are overestimated at the EU-level. Furthermore, it should be noted that the individual Member States' contributions to the estimated reductions vary greatly.

## 7.2. Model validation, assumptions and data uncertainty

### 7.2.1. Model validation

A multi-country source attribution model at the EU level has not been attempted before. In a previous report supporting an EFSA opinion on *Salmonella* in slaughter and breeder pigs, a qualitative assessment was done to estimate the role of pig meat as a source of human salmonellosis in EU (EFSA, 2010f). The assessment was based on a descriptive comparison of the serovar distribution in different animal-food source and humans, and was therefore based on very similar data as applied in the present model. The report concluded that eggs from laying hens were considered the most important source followed by pigs and broilers. Turkeys were assessed to have a relatively low impact, primarily due to a much lower consumption. For pig meat, the authors "guesstimated" that 10-20% of all salmonellosis cases could be attributed to pigs, but that the relative importance varied between MSs depending on differences in prevalences, consumption patterns and preferences, and animal and food production systems (Snary et al., 2010).

Another attribution study (Pires et al., 2010, which is described in section 5.1.2.) using EU outbreak data from 2005 and 2006 also concluded that eggs appeared to be the most important source of human salmonellosis constituting around 32% (95% CI: 21-47%) followed by "meat and poultry" (i.e. no distinction between the two sources) (11% ; 95% CI: 4-22%). Chicken, pig and turkey meat were estimated to constitute around 1.8%, 0.72% and 0.04%, respectively, but it should be noted that some cases associated with these sources were contained in the "meat and poultry" group depending on how the countries chose to report. It should also be noted that this study included a rather large proportion (42%) of cases with unknown source of infection. If these are disregarded, the relative proportion of the other sources would increase proportionally and the proportion of cases associated with eggs, "meat and poultry" and chicken would increase to 55%, 19% and 3%, respectively.

The results from the model developed as part of this opinion is in reasonable good accordance with the conclusions from the studies described above even when considering that the data used are not the same. The generally lower estimates found in the outbreak study can at least to some extend be explained by the fact that more sources were included in this study thereby reducing the relative importance of all sources.

Denmark and the Netherlands has for many years used microbial subtyping to estimate the relative importance of different sources to human salmonellosis. Comparing these national estimates with the MS-specific estimates coming out of the model in this opinion is therefore also a way of validating the model results. For Denmark, the model used in this opinion estimates that for the human cases reported in Denmark, 8% and 16% can be attributed to broilers and eggs, respectively. Data from Denmark (see Table 4) shows that the same estimates based on the national model in the period 2005-2009 are 9.5% for broilers and 6.4% for eggs. The somewhat lower estimate for eggs may be explained by the fact that the Danish model does not consider the impact of imported eggs, whereas this is included in the present model through the use of trade data.

For the Netherlands, the present model estimates that 5% and 38% of cases can be attributed to broilers and eggs, respectively. Compared with the estimates given in Table 4 (11% for broilers and 28% for eggs), there are some discrepancy, although both models agree that eggs are the more important source. One explanation may be that the Dutch model also applies phage typing to distinguish between the different sources thereby increasing the discriminatory power.

In all circumstances, it should be emphasised that direct comparison of the results of this model with that of national models is difficult because the data used and the number of sources included are different. The present model only includes the sources for with we have comparable data at the EU level, whereas the Danish and Dutch models also include cattle/beef. In addition, the Danish model have specific data on the subtype distribution in imported food (i.e. broilers, turkeys, pork and beef),

whereas the present model assumes that the serovar distribution in exported food is similar to overall national production, which may also lead to different attribution estimates for imported food.

### 7.2.2. Assumptions

A detailed description of the assumptions made for the above listed key assumptions is presented in the report of the contractor, together with the results of an assumption assessment carried out among the Experts participating in the ad hoc working group that drafted the Scientific Opinion (VOSE, 2011).

### 7.2.3. Data uncertainty

Beyond the statistical uncertainty inherent to the results provided by the model (and represented by the credibility intervals linked to each of the estimated mean values), further uncertainty arises from the different datasets used in the model, particularly related to the data quality.

The description of the data uncertainty linked to some of those datasets is implicitly included in previous sections of this Scientific Opinion and in the EFSA Report (VOSE, 2011), but it may be worth to briefly summarise and address here data uncertainties and data quality issues that may effect the model results:

***a) Salmonella in animal-food sources.***

The model includes only data on sources for which there exist comparable data of reasonably good quality for the majority of MSs i.e. Baseline Surveys data or EU harmonised monitoring data. It is assumed that these represent all the important sources of human salmonellosis, but food sources like beef, dairy products, and fruits and vegetables are not included, although they are known to act as vehicles for *Salmonella*. Omitting the cattle reservoir from the model due to lack of data, may have the consequence that a proportion of human cases were wrongly attributed to a reservoir with a similar serovar distributions i.e. pigs. Still, national attribution studies have suggested that the contribution from the cattle reservoir in general is lower than for pigs. An EU-wide baseline study of *Salmonella* in cattle or beef could be considered to investigate the role of the cattle reservoir as a source of human infections.

It is emphasised that the subtyping approach employed is tracing human infections back to the animal reservoir of origin. This means that human infections caused by fruits and vegetables contaminated with faeces from an animal reservoir would be traced back to this reservoir, which for some type of risk management decision may be appropriate.

Still, there is evidence of the importation into the EU of foodstuffs contaminated with *Salmonella* spp. (RASFF, 2010). These *Salmonella* contaminated foodstuffs may be the source of some human salmonellosis cases, and their importance are not accounted for by the model. From the results of the attribution study using outbreak data, imported food stuffs like fruits and vegetables did not seem to be contributing very much to the burden of human salmonellosis in the EU in 2005-2006.

***b) Food-borne outbreak data.***

There are differences in the level of detail in the reporting provided by the different EU MSs, which may reflect differences in the methodology and degree of outbreak-investigation carried out between MSs (new reporting systems in place in 2007). In particular, the lack of harmonisation of food categories makes it difficult to use the data for source attribution.

***c) Sporadic human salmonellosis.***

There are differences in the level of reporting provided by the different health care monitoring systems in the EU MSs, which again may reflect differences in the methodology and degree of reporting of human salmonellosis. To the extent possible, underreporting is accounted for in the model, but the estimation of underreporting factors is based on Swedish travellers data, which in itself involves some degree of uncertainty by assuming that the incidence rate among travellers returning from a particular country is the same as the overall incidence rate in the country's native population. Detailed description of the way this factor was calculated can be found in Appendix A and the EFSA Report.

***d) Level of subtyping detail.***

For the subtyping approach applied in this opinion, data on the distribution of serovars were for some of the datasets incomplete and had to be either estimated from other sources or assumed to be representative for the source in question. For the human data, where no harmonised monitoring or baseline surveys exist, this turned out to be a particular problem, which led to the exclusion of several countries. Some countries only report aggregated data i.e. total numbers with no serovar information attached, while others only report cases of *S*. Enteritidis and *S*. Typhimurium. The quality and level of serotyping also influenced the correctness of the data. For instance some countries tend to report serogroups instead of serovars thereby decreasing the comparability with other countries as well as the discriminatory power.

The above issues of serovar reporting as well as the lack of further subtyping information (e.g. phage typing) on *S*. Entertidis and *S*. Typhimurium may have resulted in attribution of some human cases to the wrong source. For instance, phage typing of *S*. Enteritidis would most likely have resulted in a better distinction between the broiler and the laying hen reservoir. Also for countries reporting only a few specific serovars or reporting mainly serogroups, human cases may have been referred to the wrong source.

***e) Serotyping quality of laboratories.***

This relates to the capacity of the laboratories to provide the correct serovar information for the *Salmonella* isolates from different sources (i.e. farm animals and humans). A ring trial perform under the auspices of ECDC in the context of the quality assurance scheme for *Salmonella* typing was carried out in March 2009 among laboratories of the Food- and Waterborne Diseases and Zoonoses network (ECDC, 2010). Of the 28 laboratories from the EU and the European Economic Area participating in this ring trial, twelve laboratories failed to correctly provide the serovar name of all the 20 strains submitted for serotyping. This means that certain degree of error in the serotyping of the strains may occur at laboratory level, and that this would be reflected in the serovar allocation of the positive *Salmonella* isolates reported in different datasets.

***f) EU production and trade data.***

EUROSTAT sourced data shows differences in the data provided by the different MSs through the different datasets (i.e. intra-community trade data, production data). Detailed description of the possible implication this may have and the way these data was considered in the model can be found in the EFSA Report (VOSE, 2011). Overall, it has to be noted that trade data refers to poultry rather than broilers (*Gallus gallus*) in particular.

In summary, the EU-wide baseline survey data in general are considered valid and provide the best available data for comparison between countries. The main issues are that not all MSs participated in all surveys, that the surveys differ in time and that some surveys are quite old. Data reported as part of the EU monitoring in broiler and laying flocks suffers from the fact that even though the minimum requirement for the monitoring is harmonised, the NCPs still differs with respect to e.g. sampling

frequencies and the detail with which the serovar distribution is reported. Finally, there exist a number of potential sources (e.g. cattle/beef), where good quality data are lacking and these sources are consequently not included in the model as described previously.

The human data are considered to be the most problematic due to the lack of harmonised monitoring. Besides the differences in degree of underreporting, the reporting of serovars also differs with respect to level of detail (e.g. some countries only report totals, some only report case numbers for *S*. Enteritidis and *S*. Typhimurium and others report serogroups).

The main factors contributing to the uncertainty of the model results beyond the statistical uncertainty are the lack of harmonised monitoring of human salmonellosis in the EU as well as the different levels of serovar detail reported in both the human and animal food source data. These uncertainties could not be statistically quantified with the model employed to support this Scientific Opinion.

More comparable *Salmonella* subtyping data from both human and animal-food from all EU Member States should be made available for more accurate future modelling and trend analysis. This could be done by means of mandatory harmonised testing programmes or centralised testing and reporting of isolates collected in Member States. The subtyping modelling approach should be repeated on a regular basis (i.e. every 3 to 5 years) in order to follow the progress of *Salmonella* control and the trends in the sources of human salmonellosis.

## CONCLUSIONS RECOMMENDATIONS

### CONCLUSIONS

#### General Conclusions

- In EU, the overall reported incidence of human salmonellosis has been decreasing from 2006 to 2009, which is mainly explained by a downward trend in the number of *Salmonella* Enteritidis infections presumably as a result of an improved surveillance and control of *S*. Enteritidis in breeding hens and laying hens in many MSs.

- In contrast, the absolute reported incidence of human *Salmonella* Typhimurium infections has increased indicating that one or more sources of these infections are increasing in importance.

- It is estimated that there are approximately 6 (90% CI: 1-15) million cases of clinical salmonellosis per year in the EU27. The disease burden of salmonellosis and its sequelae is 0.23 (90% CI: 0.05-0.6) million disability-adjusted life years (DALYs) per year and total annual costs are 2 (90% CI: 0.3-4) billion EURO.

- *Salmonella* Enteritidis and *Salmonella* Typhimurium are considered of paramount public health significance. Together, they account for approximately 75% of all serotyped human isolates. Other serovars constitute less than 2% on an individual basis.

- Any serovar that is not animal host-specific is considered capable of causing gastro-intestinal illness of varying severity in humans, and thus should be considered of potential public health significance. Nevertheless, temporal associations between some serovars and particular animal reservoir may occur, which impact the frequency of human illness and consequently may affect food safety decision making.

- Based on the results of the source-attribution model employed (named the 'Broiler Target *Salmonella* Attribution Model' or BT-SAM model) that considered the prevalence of *Salmonella* in broiler flocks as per the 2005-2006 Baseline Survey, 2.4% (95% CI: 1.8-3.4) of all human salmonellosis cases (i.e. estimated true number of cases when accounting for underreporting) in the EU were attributed to broilers.

- Around half of the broiler-associated human salmonellosis cases were caused by serovars other than the currently regulated serovars *Salmonella* Enteritidis and *Salmonella* Typhimurium.

- *Salmonella* Enteritidis and *Salmonella* Infantis constituted 42% and 23% of all broiler-associated cases respectively. *Salmonella* Hadar, *Salmonella* Typhimurium, *Salmonella* Kentucky and *Salmonella* Virchow constituted individually between 4% and 5% of all broiler-associated cases. Other serovars constituted less than 4% on an individual basis.

- For the other *Salmonella* sources included, the model estimated that around 65% (95% CI: 63-67), 28% (95% CI: 27-30) and 4.5% (95% CI: 4-5) of the estimated number of human salmonellosis cases could be attributed to laying hens (eggs), pigs and turkeys, respectively.

- The results of the model indicate that the majority of the *Salmonella* Enteritidis infections are related to the laying hen reservoir (i.e. consumption of eggs), whereas *Salmonella* Typhimurium infections originate primarily from the pig reservoir.

- Some *Salmonella* reservoirs (e.g. cattle) were not included in the model due to lack of data. It is therefore likely that the contribution of the human salmonellosis cases allocated to the animal reservoirs included in the model have been overestimated, in particular in the case of *Salmonella* Typhimurium.

- The current National Control Programmes on breeding hens and production flocks appear to have already achieved a significant reduction in the number of broiler-associated human cases.

- Complete harmonisation of the EU statutory monitoring of *Salmonella* in broiler flocks is not expected to be fully accomplished since the National Control Programmes may vary with regard to sampling methods and frequency even if the minimum requirements for the monitoring and reporting are fulfilled.

**Answers to the Terms of Reference**

The answers to the Terms of Reference are based on the results employing the source-attribution model (named the 'Broiler Target Salmonella Attribution Model' or BT-SAM model), and are referring to the broiler-associated human salmonellosis cases only (i.e. 2.4% of the total human salmonellosis cases).

- Considering the prevalence of the 23 *Salmonella* serovars included in the BT-SAM model to be as reported by the Member States in 2009, an estimated reduction of 69% (95% CI: 62-76) in the number of broiler-associated human salmonellosis cases compared to the situation in 2006 is expected.

- Considering that the prevalence of *Salmonella* Enteritidis and *Salmonella* Typhimurium is as reported by the Member States in 2009 (but keeping the prevalence for the other 21 serovars as per the 2005-2006 Baseline Survey in broiler flocks), an estimated reduction in the number of broiler-associated human salmonellosis cases of 26% compared to the situation in 2006 is expected.

- Considering that the current target of the EU control programme of *Salmonella* in broiler flocks would be met (i.e. the combined prevalence of *Salmonella* Enteritidis and *Salmonella* Typhimurium being 1% or less), and keeping the prevalence for the other 21 serovars as per the 2005-2006 Baseline Survey in broiler flocks, an estimated reduction in the number of broiler-associated human salmonellosis cases of 25% compared to the situation in 2006 is expected.

- Considering that an EU-wide target of maximum of 1% of flocks remaining positive for the all the *Salmonella* serovars considered in the model would be met, an estimated reduction in the number of broiler-associated human salmonellosis cases of 93% compared to the situation in 2006 is expected.

- The EU statutory monitoring in the Member States is likely to have a lower sensitivity in detecting positive flocks than the conducted EU-wide Baseline Surveys. For this reason, the estimated reductions in number of human salmonellosis cases are overestimated at the EU-level. Furthermore, it should be noted that the individual Member State contributions to the estimated reductions vary greatly.

- The main factors contributing to the uncertainty of the model results beyond the statistical uncertainty are the lack of harmonised monitoring of human salmonellosis in the EU as well as the different levels of serovar detail reported in both the human and animal food source data. These uncertainties could not be statistically quantified with the model employed to support this Scientific Opinion.

## RECOMMENDATIONS

- Maintaining stringent targets and controls at the EU level for *Salmonella* Enteritidis and *Salmonella* Typhimurium in broiler flocks is recommended.

- Close monitoring of the serovar distributions in humans and broilers should be strengthened to identify the emergence of serovars of public health significance.

- Targeted control of other *Salmonella* serovars in broiler flocks should be guided by the level of their occurrence in individual EU MSs.

- If sufficient information becomes available to point at particular clones of special public health significance (e.g. clones with high virulence or resistance towards antimicrobials deemed critically important for empirical treatment of human infections, but not necessarily related to particular serovars), the inclusion of such clones as part of the EU-wide targets should be considered. This will, however, require that MSs are able to apply harmonised and standardised methods in order to identify these clones unambiguously.

- In order to assess the progress on *Salmonella* control implemented in broiler flocks in all Member States in the most accurate way, the Baseline Survey performed in 2005 and 2006 should be repeated.

- An EU-wide baseline survey of *Salmonella* in cattle or beef could be considered to investigate the role of beef as a source of human infections.

- More comparable *Salmonella* subtyping data from both human and animal-food sources from all EU Member States should be made available for more accurate future modelling and trend analysis. This could be done by means of mandatory harmonised testing programmes or centralised testing and reporting of isolates collected in Member States.

- The establishment of active surveillance of human salmonellosis in all Member States is recommended, including harmonised typing of human *Salmonella* isolates and efforts to quantify the level of under-ascertainment and underreporting.

- The subtyping modelling approach should be repeated on a regular basis (i.e. every 3 to 5 years) in order to follow the progress of *Salmonella* control and the trends in the sources of human salmonellosis.

## DOCUMENTATION PROVIDED TO EFSA

1. Letter (Ref. SANCO/E2/KDS/ca D(2008) 520108 dated 02 April 2008) from the European Commission regarding a request quantitative estimations of the public health impact of setting an new target for the reduction of *Salmonella* in certain poultry populations.

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# APPENDICES

## A. AN ESTIMATION OF THE UNDERREPORTING FACTOR FOR HUMAN SALMONELLOSIS IN THE EU BASED ON DATA FROM SWEDISH TRAVELLERS

### 1. Data and methodological approach

In 2009 there were approximately 110,000 notified cases of salmonellosis in the EU. Table 1 provides a summary of the reported data. The EU-average incidence rate of reported cases was 23.7 cases per 100,000 population (EFSA and ECDC, 2011).

**Table 1:** Reported confirmed human salmonellosis in the EU, Norway and Switzerland, 2009 (modified from EFSA and ECDC, 2011).

| Country | Population[1] (million) | Reported human salmonellosis Cases | Incidence rate (per 100.000) |
|---|---|---|---|
| Austria | 8.355 | 2,775 | 33.2 |
| Belgium | 10.753 | 3,113 | 29.2 |
| Bulgaria | 7.607 | 1,247 | 16.4 |
| Cyprus | 0.797 | 134 | 16.8 |
| Czech Republic | 10.468 | 10,480 | 100.1 |
| Denmark | 5.511 | 2,130 | 38.6 |
| Estonia | 1.34 | 261 | 19.5 |
| Finland | 5.326 | 2,329 | 43.7 |
| France | 62.469 | 7,153 | 11.1 |
| Germany | 82.002 | 31,395 | 38.3 |
| Greece | 11.26 | 403 | 3.6 |
| Hungary | 10.031 | 5,873 | 58.2 |
| Ireland | 4.45 | 335 | 7.5 |
| Italy | 60.045 | 4,156 | 6.9 |
| Latvia | 2.261 | 795 | 35.2 |
| Lithuania | 3.35 | 2,063 | 61.6 |
| Luxembourg | 0.494 | 162 | 32.8 |
| Malta | 0.414 | 124 | 30 |
| Poland | 38.136 | 8,521 | 22.3 |
| Portugal | 10.627 | 220 | 2.1 |
| Romania | 21.499 | 1,105 | 5.1 |
| Slovakia | 5.412 | 4,182 | 77.3 |
| Slovenia | 2.032 | 616 | 30.3 |
| Spain[2] | 45.828 | 4,304 | 37.6 |
| Sweden | 9.256 | 3,054 | 33 |
| The Netherlands[3] | 16.486 | 1,205 | 11.4 |
| United Kingdom | 61.595 | 10,479 | 17 |
| EU-27 | 497.805 | 108,614 | 23.7 |
| Norway | 4.737 | 1,235 | 25.7 |
| Switzerland | 7.701 | 1,325 | 17.2 |

1. Population data supplied by ECDC, except for Switzerland that was provided by EFSA Unit on Zoonoses data collection
2. Notification rates calculated with estimated population coverage of 25%
3. Sentinel system: notification rates calculated with estimated population coverage of 64%

As discussed in a previous Opinion of EFSA (2009), there is considerable underascertainment and underreporting and the true incidence of human salmonellosis was estimated to range between 1 and 15 million cases per year. There is little information on specific underreporting factors in different MSs. In order to provide appropriate input data for the attribution model, country-specific estimates of the true incidence of salmonellosis are required.

For the purpose of the current Opinion, the estimates on the true incidence were based on data describing differential risks to Swedish travellers as published originally by de Jong and Ekdahl (2006). Updated information on the risk for Swedish travellers in the EU, as presented in Table 2, were obtained from the Swedish Institute for Communicable Disease Control (Smittskyddsinstitutet, SMI, Solna, Sweden, epidemiologist Sofie Ivarsson). Cases reported in the Table 2 were enumerated in the Swedish infectious disease surveillance system (SmiNet) and covered the years 2005-2009. Information on whether a case was travel-related or not, and the country of travel, was available for 98-99%% of all reported cases with salmonellosis. Data on travel patterns of Swedish residents was obtained from the Swedish Travel and Tourism Data Base (TDB, Resurs AB, Stockholm, Sweden). More information on the survey methods and data processing is available at http://www.resursab.se/. Only travels with an overnight stay in the country of destination were included in the model.

A total of 3,854 cases with salmonellosis and a history of foreign travel before disease onset were registered and approximately 46 million travels were undertaken by Swedes. The average number of travels per year was 9.2 million, higher than reported for 1997-2003 (7.5 million). Nevertheless, the number of reported cases of salmonellosis decreased from 1983 to 771 per year. The average risk (per 100,000 travels) of salmonellosis in Swedish travellers returning from the EU in 2005-2009 was 8.44 (90% c.i. 8.22-8.67), ranging between 0.13 per 100,000 for travellers returning from Finland to 94.26 for travellers returning from Bulgaria.

Uncertainty in the risk estimate was simulated by bootstrapping, assuming a Poisson process. Monte Carlo simulation were performed using @RISK 5.0 (Palisade Corporation, Ithaca, NY, USA), and add-in to Microsoft Excel®. Note that this approach takes only the sampling effects in case numbers into account. In the paper by de Jong and Ekdahl (2006) uncertainty is estimated by a lognormal approximation, also taking sampling effects in the travel data into account. Such information was not currently available. However, bootstrapping of data as reported in Ekdahl and Giesecke showed only slightly lower uncertainty margins for the bootstrap approach. Apparently, uncertainty is dominated by sampling effects in the case numbers.

**Table 2:** Risks of salmonellosis in returning Swedish travellers, 2005-2009.

| Risk to Swedish travelers | | | |
|---|---|---|---|
| *Country* | *Cases* | *Journeys* | *Risk per 100,000 travels* |
| Austria | **27** | **924.831** | **2,92** |
| Belgium | **5** | **620.008** | **0,81** |
| Bulgaria | **482** | **511.366** | **94,26** |
| Cyprus | **138** | **592.149** | **23,30** |
| Czech Republic | **151** | **652.565** | **23,14** |
| Denmark | **93** | **6.884.980** | **1,35** |
| Estonia | **30** | **1.135.989** | **2,64** |
| Finland | **10** | **7.669.097** | **0,13** |
| France | **63** | **2.637.364** | **2,39** |
| Germany | **155** | **5.176.651** | **2,99** |
| Greece | **823** | **2.339.178** | **35,18** |
| Hungary | **157** | **501.369** | **31,31** |
| Ireland | **1** | **308.430** | **0,32** |
| Italy | **106** | **2.668.173** | **3,97** |
| Latvia | **72** | **577.638** | **12,46** |
| Lithuania | **33** | **113.304** | **29,13** |
| Luxembourg | **1** | **84.460** | **1,18** |
| Malta | **84** | **157.247** | **53,42** |
| Poland | **185** | **907.010** | **20,40** |
| Portugal | **202** | **585.442** | **34,50** |
| Romania | **13** | **90.340** | **14,39** |
| Slovakia | **17** | **51.691** | **32,89** |
| Slovenia | **9** | **92.058** | **9,78** |
| Spain | **948** | **5.889.338** | **16,10** |
| Sweden | **NA** | **NA** | **NA** |
| The Netherlands | **12** | **780.458** | **1,54** |
| United Kingdom | **37** | **3.702.100** | **1,00** |
| EU-27 | **3.854** | **45.653.238** | **8,44** |
| Norway | **12** | **4.916.615** | **0,24** |
| Switzerland | **5** | **508.912** | **0,98** |

NA: Not applicable
There were no cases observed in Ireland and Luxembourg. For the model, 1 observed case was inserted.

Estimates of the true incidence were anchored to population-based estimates from the Netherlands, based on raw data from a Dutch cohort study (De Wit et al., 2001), where the incidence rates from these studies were applied to the population of 2009 and scaled to the observed average of laboratory-confirmed cases for these years in comparison to the year 1999 when the cohort study was performed (a decrease of 45%). A full description of the simulation method is given in Havelaar et al. (2004), who used this approach for estimating STEC O157-associated gastro-enteritis in the Netherlands. 31,700 (90% CI: 6,500-78,600) cases of salmonellosis were estimated to occur in the Netherlands in 2009, an incidence rate of 192/100,000 person years.

The incidence rate in other MSs was calculated from this estimate as:

$$IR_C = (IR_{NL} \times ST_C) / ST_{NL}$$

Where,

$IR_C$ = incidence rate of salmonellosis in country C (the Netherlands for C= NL)

$ST_C$ = risk of salmonellosis for Swedish travellers to country C ((the Netherlands for C= NL).

The true incidence of salmonellosis in a country was estimated by multiplying with the population size in 2009, as reported by EUROSTAT. An underreporting factor can then be estimated by comparison of the estimated true incidence with the reported incidence as represented in Table 1. The % of reported cases was calculated as the inverse of the underreporting factor. The incidence rate of human salmonellosis in Sweden cannot be calculated from this data set, and was assumed to be the same as in Finland.

## 2. Results

Based on these calculations, Table 3 shows the estimated risk to Swedish travellers, the true incidence of human salmonellosis in the EU27 and the estimated underreporting factor.

**Table 3:** Estimated risk to Swedish travellers, estimated true incidence of human salmonellosis in the EU27, Norway and Switzerland and estimated underreporting factor.

| | **Swedish travellers' Risk** | | **True incidence** | | **Underreporting** | |
|---|---|---|---|---|---|---|
| **Country** | **(per 100,000)** | **(relative to NL)** | **Cases** | **Incidence rate (per 100.000)** | **factor** | **% reported** |
| Austria | 2.92 | 1.899 | 30,483 | 365 | 11.0 | 9.10% |
| Belgium | 0.81 | 0.524 | 10,750 | 101 | 3.5 | 28.96% |
| Bulgaria | 94.26 | 61.303 | 895,981 | 11779 | 718.5 | 0.14% |
| Cyprus | 23.30 | 15.157 | 23,208 | 2912 | 173.2 | 0.58% |
| Czech Republic | 23.14 | 15.049 | 302,687 | 2892 | 28.9 | 3.46% |
| Denmark | 1.35 | 0.879 | 9,303 | 169 | 4.4 | 22.89% |
| Estonia | 2.64 | 1.718 | 4,424 | 330 | 16.9 | 5.90% |
| Finland | 0.13 | 0.085 | 868 | 16 | 0.4 | 268.34% |
| France | 2.39 | 1.554 | 192,097 | 299 | 26.9 | 3.72% |
| Germany | 2.99 | 1.947 | 306,835 | 374 | 9.8 | 10.23% |
| Greece | 35.18 | 22.883 | 495,092 | 4397 | 1,228.5 | 0.08% |
| Hungary | 31.31 | 20.366 | 392,537 | 3913 | 66.8 | 1.50% |
| Ireland | 0.32 | 0.211 | 1,803 | 41 | 5.4 | 18.58% |
| Italy | 3.97 | 2.584 | 298,102 | 496 | 71.7 | 1.39% |
| Latvia | 12.46 | 8.107 | 35,223 | 1558 | 44.3 | 2.26% |
| Lithuania | 29.13 | 18.942 | 121,925 | 3640 | 59.1 | 1.69% |
| Luxembourg | 1.18 | 0.770 | 730 | 148 | 4.5 | 22.19% |
| Malta | 53.42 | 34.743 | 27,611 | 6676 | 222.7 | 0.45% |
| Poland | 20.40 | 13.266 | 972,052 | 2549 | 114.1 | 0.88% |
| Portugal | 34.50 | 22.441 | 458,231 | 4312 | 2,082.9 | 0.05% |
| Romania | 14.39 | 9.359 | 386,606 | 1798 | 349.9 | 0.29% |
| Slovakia | 32.89 | 21.389 | 222,436 | 4110 | 53.2 | 1.88% |
| Slovenia | 9.78 | 6.358 | 24,830 | 1222 | 40.3 | 2.48% |
| Spain | 16.10 | 10.469 | 921,871 | 2012 | 214.2 | 0.47% |
| Sweden | NA | 0.085 | 1,508 | 16 | 0.5 | 202.48% |
| The Netherlands | 1.54 | 1.000 | 31,677 | 192 | 26.3 | 3.80% |
| United Kingdom | 1.00 | 0.650 | 76,411 | 125 | 7.3 | 13.71% |
| **EU-27** | **8.44** | **5.490** | **6,245,281** | **1251** | **57.5** | **1.74%** |
| Norway | 0.24 | 0.159 | 1,464 | 31 | 1.2 | 84.37% |
| Switzerland | 0.98 | 0.639 | 9,456 | 123 | 7.1 | 14.01% |

For the EU-27 in 2009, the incidence of salmonellosis was approx. 6.2 (90% CI: 1.2-15) million cases, which fits well in the range reported before. Also note that for the UK, the underreporting factor is estimated at 7.3 (90% CI: 1.5-17). An independent estimate of 3.2 in the mid-1990s, based on the Infectious Intestinal Disease study (Wheeler et al., 1999) was considerably lower, although within the (wide) confidence bounds. The underreporting factor for the EU as a whole is estimated at approximately 57.5 (90% CI: 10.9-140.3), but ranged between 0.4 for Finland to more than 2,000 for Portugal.

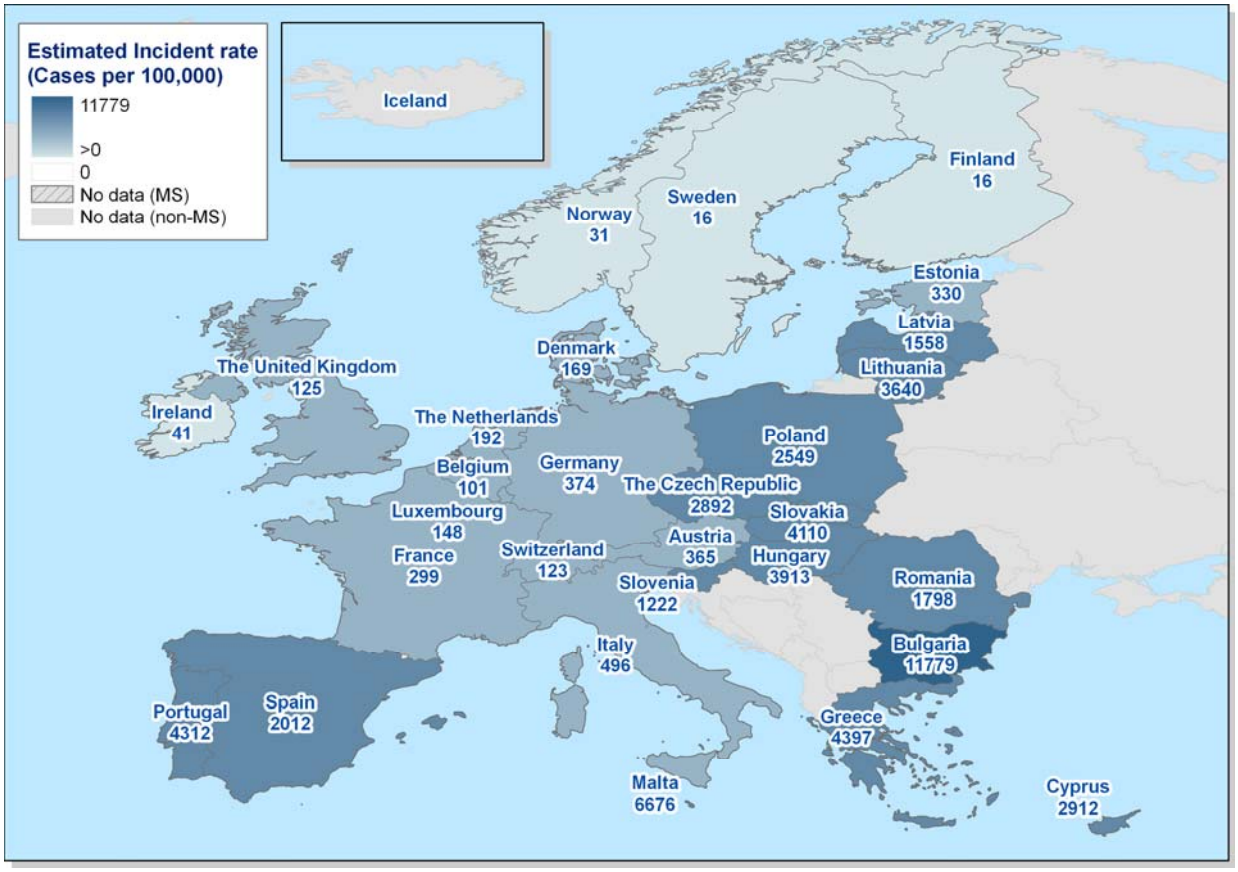


**Figure 1:** Estimated true incidence rate of human salmonellosis in the EU27, Norway and Switzerland. Figure kindly provided by the EFSA Unit on Zoonoses data collection.

These incidence estimates can be used to update a previous estimate of the EFSA Panel on Biological Hazards (EFSA, 2010) of the disease burden and costs of salmonellosis, and its sequelae. The public health impact is then estimated at 0.23 (90% CI: 0.05-1.5) million disability-adjusted life years (DALYs) per year for the EU-27 with an annual cost of about 2 (90% CI: 0.3-4) billion €. Details are presented in Table 4.

**Table 4:** Estimated cost of human salmonellosis in the EU-27 (5% confidence limit, mean value and 95% confidence limit). Updated estimates based on previous EFSA Opinion (EFSA, 2010).

| | 5% CL | Mean | 95% CL |
|---|---|---|---|
| Estimated incidence | $1.2 \times 10^6$ | $6.2 \times 10^6$ | $1.5 \times 10^7$ |
| DALYs EU-27 (0.04 DALYs per case) | $4.8 \times 10^4$ | $2.3 \times 10^5$ | $5.6 \times 10^5$ |
| Cost EU-27 (250 EURO per case) | $3.3 \times 10^8$ | $1.6 \times 10^9$ | $3.8 \times 10^9$ |

**3. Validation of results by comparing these with those of other similar studies.**

The risks to Swedish travellers in the period 2005-2009 were compared with those reported previously by De Jong and Ekdahl (2006) for the period 1997-2003, see Figure 2. Estimates were significantly correlated ($p << 0.001$), but the risks in the 2005-2009 period were approximately half of those reported before (linear regression model forced through the origin, regression coefficient 0.51).

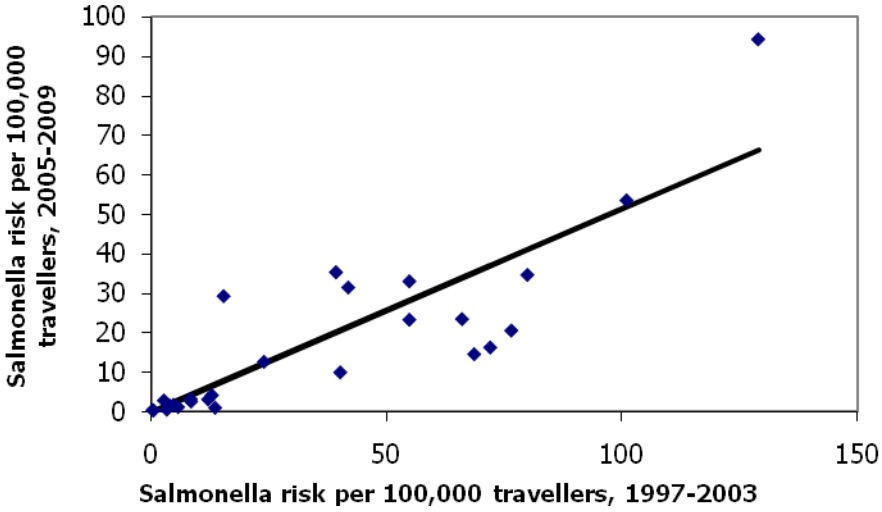


**Figure 2:** Comparison of salmonellosis risks to Swedish travellers in two time periods

Disease incidence per MS based on the 2005-2009 data was also compared to the incidence estimated by De Jong and Ekdahl (2006) for 1997-2003, see Figure 3. There was a significant correlation ($p << 0.001$) between the two risk estimates. The average incidence estimate for 2005 was 2.3 times higher than for 1997-2003. This does not imply that the incidence of salmonellosis has increased in the past years. The earlier estimates were arrived at by anchoring to the data reported by Norway, where no underreporting was assumed to occur. In contrast, the new estimates were anchored in a Dutch population based survey, so the same average level of underreporting was not expected.

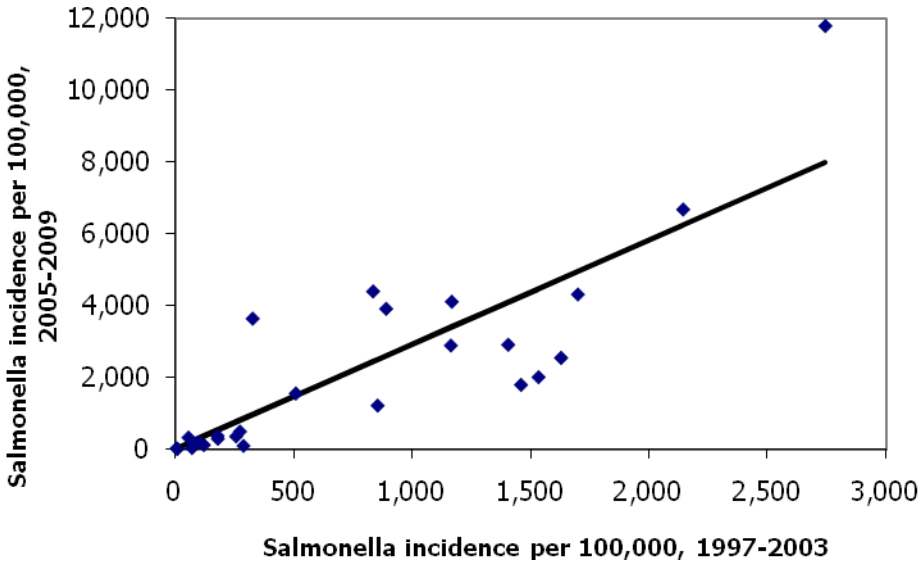


**Figure 3:** Comparison of *Salmonella* incidence in two time periods, according to two estimation methods

A further comparison can be made with serosurveillance data. Simonsen et al. (2009) reported a statistical model to estimate the seroincidence (i.e. the incidence of new infections) from cross-sectional seroprevalence data. Note that a large proportion of these infections will be asymptomatic. Using this modelling approach and existing serum collections, the seroincidence was estimated in the Med-Vet-Net Network of Excellence for 8 European countries (Falkenhorst et al., 2009 and submitted for publication). Figure 4 shows results from this study compared with the true disease incidence estimates based on Swedish travellers risks. There was a significant correlation between the two estimates (p = 0.02). The regression coefficient was 0.0034, suggesting that on average, 3.4% of all infections would lead to symptomatic illness.

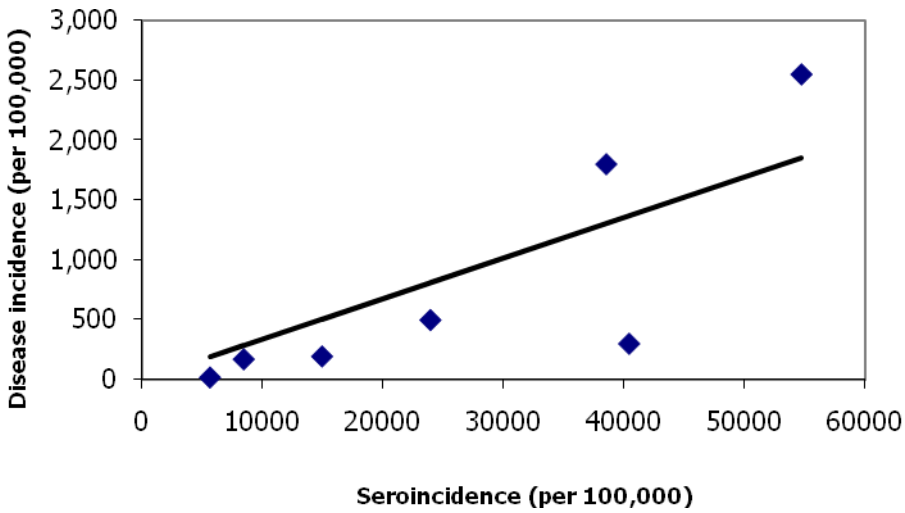


**Figure 4:** Comparison of seroincidence and disease incidence

De Jong and Ekdahl (2006) demonstrated a strong correlation between their estimates of incidence of salmonellosis in EU Member states and the prevalence of Salmonella in laying hens as found in the EU-wide baseline survey. Since the baseline study, EU-wide control programmes have been implemented, and have led to considerable decreases in the prevalence of Salmonella in laying hen flocks, even when the reduced sensitivity of the mandatory monitoring as compared to the baseline study protocol is taken into account. The new estimates were compared to more recent data on *Salmonella* prevalence in laying hens in 2008 (EFSA, 2010), see Figure 5. Disease incidence in humans was significantly correlated with *Salmonella* Enteritidis prevalence in laying hens (p = 0.02) but not with prevalence of all *Salmonella* serovars (p = 0.08).

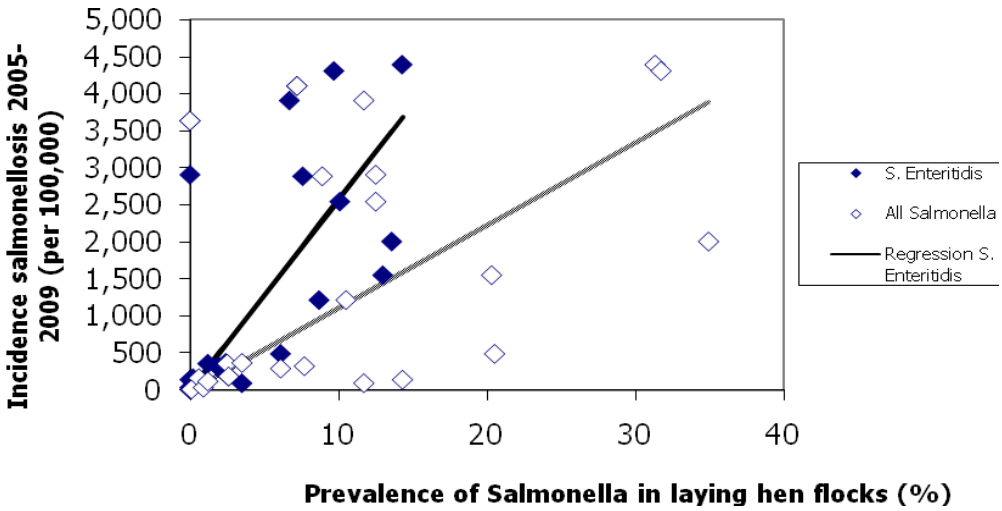


**Figure 5:** Comparison of disease incidence and prevalence of *Salmonella* in flocks of laying hens in the EU.

## 4. Assumptions and limitations

Estimated results have to be considered understanding the different assumptions and limitations behind the model and the data employed. These include:

- The case data are extracted from the Swedish infectious disease surveillance system (SmiNet) and rely on laboratories and physicians reporting diagnosed cases to SMI. Clearly, only a fraction of all cases of illness will be reported. For this model, mainly the potential of differential reporting per country should be considered.

- Both cases and travels are counted as such, without consideration of the duration of the stay abroad, or the purpose of the visit (business or leisure). Day travels were excluded from the dataset. There are, for example, a very high number of travels to Denmark, Finland and Norway, which may be mainly for business purposes and of short duration (Ekdahl and Giesecke, 2004). For these trips, the duration of exposure may be shorter but on the other hand travellers who fall ill will most likely have returned to their home country and will be reported in the Swedish public health system when seeking health care. On the other hand, trips to the Mediterranean area may be mainly for leisure purpose and last one or more weeks. Travellers may be exposed for longer time periods, but when ill may have recovered before returning home. It is difficult to predict in which direction biases may occur.

- The estimated underreporting factor for Finland is less than 1, implying that there are fewer cases than actually reported, which is highly unlikely. This might indicate that for – presumably - short-term visits, the risks to travellers may be underestimated.

- Further biases may be introduced by seasonal travel patterns. It is likely that most travels to the Mediterranean take place in summer, when the prevalence of *Salmonella* in animals and food is highest.

- Health-seeking behaviour of travellers or medical decisions about stool cultures may be affected by the country of destination.

- Another important assumption is that relative risks to Swedish travellers are predictive of risks for the local population. This assumption ignores any potential effects of acquired immunity, differences in eating habits and local residents, differences between strains circulating in different parts of Europe.

It is currently not possible to conclude on the magnitude or even the direction of these biases. A detailed discussion of potential biases in the data is provided by Ekdahl and Giesecke (2004).

## 5. Concluding remarks

The data presented above suggest a very high incidence of human salmonellosis in the EU (each year, approximately one out of every 80 inhabitants would be affected). Consequently, considerable public health benefits are expected if *Salmonella* contamination of food and food producing animals would be reduced. Nevertheless, the limitations and assumptions in the risk assessment models need to be taken into consideration when interpreting these findings.

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## B. *SALMONELLA* MONITORING PROGRAMMES IN BROILER FLOCKS (*GALLUS GALLUS*) AND ACTIONS TAKEN FOLLOWING IDENTIFICATION OF A POSITIVE FLOCK

The following tables are sourced from: EFSA (European Food Safety Authority, European Centre for Disease Prevention and Control), 2011. The European Union Summary Report on Trends and sources of Zoonoses and Zoonotic Agents and Food-borne Outbreaks in 2009.

**Table 1:** *Salmonella* monitoring programmes in broiler flocks (*Gallus gallus*), 2009.

| | |
|---|---|
| Countries running an approved monitoring and control programme[1] according to Regulation (EC) No 2160/2003 and meeting at least the minimum sampling requirements set out by Regulation (EC) No 646/2007[2] | |
| MSs with approved surveillance programme (Decision 2008/815/EC) | All |
| Non-MS with approved surveillance programmes (ESA Decision No 364/07/COL) | NO |
| MSs with EU co-financing (Decision 2008/897/EC as amended by Decision 2009/858/EC) | All MSs except FI, LT, SE |
| Countries with additional sampling | DK[3] |
| Minimum requirement according to Regulation (EC) No 2160/2003 as amended by Regulation (EC) No 646/2007 | |
| Rearing period[4] | |
| Within 3 weeks of slaughter | At least two pairs of boot/sock swabs pooled into one sample[5] |
| Diagnostic methods used | |
| ISO 6579 (2002) | CZ, EE, ES, FI, FR, GR, IT, , NO, PL, SE (faecal samples), SK, UK |
| Modified ISO 6579, Annex D | LU |
| Modified ISO 6579 (2002) | AT, CH, DE, SI |
| ISO 6579 (2002) / Amendment 1:2007 | BE, ES, FI (Flocks), LV (Flocks), RO |
| NMKL No 71:1999 | FI |
| Bacteriological culture | DK, LT, UK, IE |
| Method in accordance with the O.I.E. manual, 5th ed., 2004 | SI |

1. Non-MSs (EFTA members) must apply the EU legislation according to Decision of the EEA Joint Committee No 101/2006.
2. Regulation (EC) 646/2007 sets the Community targets for the reduction of the prevalence of certain *Salmonella* types in broiler flocks and setting the testing scheme to verify the achievement of the Community targets for S. Enteritidis and S. Typhimurium.
3. In Denmark, all flocks are tested twice during rearing at 15-21 days and 7-10 days before slaughter.
4. Once a year, the competent authority sample at least one flock on 10% of holdings comprising at more than 5,000 birds
5. Two pairs of boot/sock swabs might be replaced by one pair of boot/sock swabs and one sample of dust collected multiple places in the broiler house

**Table 2:** Measures taken in broilers (*Gallus gallus*) in case of *Salmonella* infections, 2009.

| Control measures | Countries |
|---|---|
| Serovars covered | |
| All Serovars | BE, DK, FI, LT, LU, NO, NL, SE[1] |
| *S.* Enteritidis and *S.* Typhimurium | AT, BG, CH, EE, ES, FR[2], IE, LV, RO, SI, SK, UK[3] |
| Restrictions on the flock | |
| Immediately following suspicion | DK, EE, FR, LU, NO, NL, RO, SI, SE |
| Consequence for the flock | |
| Slaughter | SK |
| Slaughtered and heat treated | CH, DK, FI, LT, LU, LV, NO, SI |
| Sanitary slaughter | AT, BE, IE, NL, UK |
| Destruction | FI, FR, LV, SE |
| Slaughter or destruction | BG, EE, IE, SK, UK |
| Other consequence | |
| Feedingstuffs are restricted (heat treatment or destruction) | EE, LU, NO, SE |
| Disposal of manure restricted | EE, FI, NO, SK, SI, SE |
| Cleaning and disinfection | |
| Obligatory | AT, BE, BG, CH, DK, EE, ES, FI, FR, LT, LV, NO, NL, SI, SE, ES, FR, SE |
| Negative bacteriological result required before restocking | AT, BE, BG, CH, CZ, DK, EE, ES, FI, FR, LU, NL, NO, RO, SI, SE |
| Requirement of an empty period | AT (14 days), EE (21 days), LU (21 days), NO (30 days after disinfection), DK, ES (12 days) |
| Further investigations | |
| Epidemiological investigation is always started | CZ, EE, ES, FI, FR, IE, LU, NO, SE, SK, UK |
| Feed suppliers are always included in the investigation | AT, EE, FI, IE, LU, NO, NL, SE |
| Contact herds are included in the investigation | EE, FI, FR, LU, NO, SE |
| Breeding flock that contributed to the hatch will be traced | AT, FI, FR, IE, LU, NO, NL, UK, SE |
| Vaccination | |
| Permitted | AT, CZ, EE[4], FR, LT, LU, LV, SI, SK, UK |
| Vaccine not registered | AT, BE, DK, ES |
| Prohibited | CH, FI, NO, SE |

1. In Sweden, for invasive serovars and non-invasive serovars different control strategies may be applied but are not used in practice
2. In France, all isolation of *Salmonella* spp. must be reported
3. In the United Kingdom, all isolations of *Salmonella* spp. must be reported
4. In Estonia, vaccination against *Salmonella* could only be performed based on the Veterinary and Food Board approval

## C. THE BROILER MEAT PRODUCTION CHAIN

Various poultry species, mainly chickens (*Gallus gallus*), turkeys, and guinea fowl, are used in industrial meat production, and their importance varies with regions and food consumption habits.

In 2008, 11.6 millions tons (Tons Equivalent Carcasses) of poultry meat were produced in the EU, comprising mainly broilers (75%), turkeys (16%) and ducks (4 %). Poultry meat is the second most important meat species produced in the EU and, at 13%, the EU is the third highest poultry meat producer in the world. Nevertheless, since 2007, the importation of poultry meat from third countries is increasing, whereas the export is slowly decreasing since 2002. In 2008, the amount of import and export of poultry meat was quite similar (app. 800,000 tons) , reaching a self-sufficient level.

Production of poultry meat is based on selection of pure lineages of male and female birds using very precise genetic criteria, including productivity (growth rate), quality of products and resistance against disease. The selection methods assure a uniform quality of bird for further multiplication and production. Selection criteria differ according to the type of production. There are also different genetic lines of birds for conventional and free-range or organic production systems.

Most birds are raised in closed ('intensive') systems. Some alternative poultry management systems also exist, such as organic and free-range production.

In a conventional flock, the birds are kept inside the houses. A free-range flock system is a flock production type where the birds have access to outside. An organic flock system is a production type that is similar to the free range system and that fulfils the requirements set for organic production; birds have access to outside and are registered with a recognised Organic Standard Regulatory Organisation.

Management systems, which incorporate a free-range phase within the rearing period vary widely over Europe in terms of period of free-ranging, conditions and stages prior to free ranging, but the biosecurity measures adopted for the rearing phase, especially in growing units tend to be different due to outdoor access. Much of this variation relates to national management practices. However, for all free-range flocks chicks are placed first in an indoor containment before free-range release.

In general, day old chicks are reared in specific houses ; the materials (e.g. feeders, drinkers) and the environment of the rearing house (e.g. walls, grounds) are cleaned and disinfected before the arrival of chicks. Normally it is recommended to put all chicks coming from the same hatchery (same genetic line) together the same day to constitute a flock.

During the rearing period, broilers are fed mainly with heat treated feed coming from industrial feed mills. Home-mixed rations are rarely used for fast-growing meat because these birds require a higher nutrient density and well-balanced diet to achieve maximum economic growth and health potential (EFSA, 2008).

Beyond the daily sanitary and technical surveillances of the flock, other punctual interventions can occur during the rearing period. Among these, depopulation (thinning) consists of removing part of the flock before the end of the rearing period. This procedure follows 2 main objectives:

a. Thinning during the rearing period (4-5 weeks) allows increased weight gain in remaining birds. The Council Directive 2007/43/EC laying down minimum rules for the protection of chickens kept for meat production, proposes a maximum stocking density in a holding or in a house of a holding lower than 33 kg /$m^2$ (39 kg/$m^2$ conditionally). The implementation of this regulation probably encourages thinning of birds during the rearing period.

b. For logistic reasons due to the further slaughter operations, a depopulation can be done mainly during the last week of the rearing period, allowing the slaughter of the flock within 2 or 3 days.

This operation induces a lot of movements inside the house (e.g. workers, material) increasing the risk of biological contamination. Consequently these interventions must follow strict hygienic and sanitary measures.

Before transport of birds, feed should be withdrawn in order to reduce defecation during transport and to facilitate the evisceration of birds in the processing plant (empty intestine). Council Directive 2007/43/EC limits feed withdrawal to maximum 12 hours before the expected slaughter time.

The slaughter age for broilers varies considerably in the EU: minimum 20 days, maximum 150 days, and mean 41.4 days in the baseline survey (EFSA, 2007). Many producers aim for heavy carcasses and consequently increase the age of slaughtering. In organic and some free range production, the age should not be lower than 81 days (Regulation (EC) No 889/2009).

The transport of live birds from the farm to the slaughter plant is very diverse according to the processing company strategy. Currently birds are transported in crates, containers, or cages. Crates and containers can be introduced in the poultry house and then loaded directly, but cages should be loaded outside. Regulation (EC) No 853/2004 indicates only some requirements concerning the transport of live animals: (1) animals must be handled carefully without causing unnecessary distress; (2) Crates must be easy to clean and disinfect, and all equipment must be cleaned, washed and disinfected immediately after emptying and, if necessary, before re-use. Another requirement concerns animal showing symptoms of disease or originating in flocks known to be contaminated with agents of public health importance: these birds may only be transported to the slaughterhouse when permitted by the competent authority.

Concerning the slaughtering step, different techniques can be used depending mainly on the commercial strategy of the company. In general, birds are put on the line and stunned by use of electrical or gas stunning, followed by bleeding using neck-cutting. The carcasses are processed by use of machinery on a production line at speeds of up to 12,000 carcasses per hour. The feathers are first loosened by scalding the carcasses in warm water, and then removed by use of rotating rubber fingers leaving the skin intact. The water temperature in the scalding tank varies between 52 and 59˚C depending if the product will be refrigerated or frozen. Different techniques (e.g. soaking, spraying, steam) can be used for scalding carcasses, but soaking seems to be the most effective, especially by using a multi stage set up associated with a counter current flow reducing cross contamination. After removing the head and feet, the viscera are extracted manually or using a series of machines followed, if necessary, by washing the carcasses in an 'inside/outside washer'. After inspection and evisceration, carcasses must be chilled to not more than 4°C as soon as possible. The chilling operation can be by immersion in cold water or, most commonly in the EU, by forced cold air (dry or with intermittent water sprays). Water chilling is mainly used for frozen and air chilling for refrigerated products.

In smaller abattoirs and local small scale production, manual handling would replace most of the automated process. Thus, practices like plucking or evisceration may be performed more manually.

After slaughter, broiler carcasses may be marketed whole or as cuts and portions, or as further processed products, which offer convenience to consumers. As certain products and cuts, such as boneless, skin-less breasts, are becoming popular with consumers in certain countries, large factories use automated cutting and processing equipment, while smaller operations employ manual cutting procedures. Cutting of broiler carcasses involves removal of legs, wings and the breast, while the legs and leg quarters may be further cut into thighs and drumsticks. The wings can also be cut into drumettes, while the remaining carcass racks are used for production of soup stock, pet food, or waste. Portioning and sizing are needed for situations where uniform portions of meat are served. In addition to deboning, cutting, portioning and packaging, further processing involves operations such as tumbling, massaging, reforming, emulsifying, breading or battering, marinating, and partial or

complete cooking. Forming is done by reducing the size of meat cuts, mixing with brines containing binding and flavouring ingredients, tumbling to increase penetration of brine, and forming or moulding into appropriate sizes and shapes with extruder/stuffers. In general, further processing of broiler carcasses may be as simple as splitting the carcass into halves or as complex as producing marinated or breaded, partially or fully cooked products (Mead, 2000).

**Concluding remarks**

- UE is the third highest poultry meat producer in the world. Nevertheless the importation of poultry meat from third countries is increasing.
- The industrial boiler production is very diverse: most birds are raised in closed ('intensive') systems but some alternative poultry management systems also exist, such as organic and free-range production.

**References Appendix B**

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## D. REVIEW OF DATA ON CONSUMPTION OF BROILER MEAT IN THE EU

In Table 1 poultry consumption data extracted from the FAOSTAT and EUROSTAT databases are presented. Also, the data published in the annual report 2009 from the Association of Poultry Processors and Poultry Trade in the EU Countries (AVEC, 2010[1]) are shown. These are based on different data sources including the EUROSTAT and various national statistics. Although, there is an overall good agreement between the different data sources, there are some countries for which there is discrepancy between the different data sources even if the reporting year is the same. This is presumably due to different data collection and reporting methods, as well as different national bodies are conducting the reporting. Figure 8 (section 5.2 main body) was created using the FAOSTAT data as this dataset provides data for all MSs for 2007 and is assumed to be the most comparable of the three datasets presented. As expected the consumption between MSs varies considerable, from 13 kg poultry meat per capita per year in Greece to 39 kg in Luxembourg, however for both these countries it should be noted that there is discrepancy between what is reported to FAOSTAT and EUROSTAT and the data should be interpreted with care. In the EU as a whole, the mean consumption was 20 kg per capita per year in 2007 (FAOSTAT, accessed 27/02/11). From the EUROSTAT dataset and the AVEC report, it also appears that the consumption of poultry meat for most MSs has been relatively stable over the last five years (FAOSTAT, accessed 27/02/11; AVEC, 2010).

**Table 1:** Consumption of poultry meat in the European Union (FAOSTAT, accessed 27 February 2011); EUROSTAT, accessed 27 February 2011; AVEC, 2010[13]).

| | FAOSTAT | EUROSTAT | | AVEC Annual Report for 2009 | | | | |
|---|---|---|---|---|---|---|---|---|
| | kg poultry meat/capita/yr (2007 data) | kg poultry meat/capita/ yr | Most recent year of reporting | kg poultry meat/capita /yr | Most recent year of reporting | kg broiler meat/capita/ yr | % broiler /poultry | Year of reporting |
| Austria | 17 | 20 | 2009 | 18.5 | 2008 | 11.7 | 63 | 2007 |
| Belgium | 25 | 23 | 2005 | 18.7 | 2008 | - | - | - |
| Bulgaria | 20 | 22 | 2009 | - | 2008 | - | - | - |
| Czech Republic | 24 | 2 | 2002 | 23.8 | 2008 | - | - | - |
| Denmark | 18 | 26 | 2009 | 24 | 2008 | - | - | - |
| Germany | 15 | 18 | 2007 | 18.8 | 2008 | 11.1 | 59 | 2008 |
| Estonia | 17 | 21 | 2009 | 17 | 2005 | - | - | - |
| Ireland | 25 | 27 | 2007 | 32.2 | 2008 | - | - | - |
| Greece | 13 | 30 | 2007 | 20.5 | 2008 | - | - | - |
| Spain | 27 | 32 | 2004 | 30.5 | 2008 | - | - | - |
| France | 21 | 23 | 2009 | 24.5 | 2008 | 13.5 | 55 | 2008 |
| Italy | 15 | 15 | 2006 | 17.5 | 2008 | 11.1 | 63 | 2008 |
| Cyprus | 33 | 45 | 2007 | 38.4 | 2004 | - | - | - |
| Latvia | 20 | 5 | 2003 | 20.2 | 2008 | - | - | - |
| Lithuania | 25 | 12 | 2002 | 23.3 | 2008 | - | - | - |
| Luxembourg | 39 | 15 | 2009 | - | 2008 | - | - | - |
| Hungary | 27 | 29 | 2009 | 31.7 | 2008 | - | - | - |
| Malta | 24 | 23 | 2003 | 25.3 | 2004 | - | - | - |
| Netherlands | 14 | 22 | 2007 | 22.5 | 2008 | 18.4 | 82 | 2008 |
| Poland | 20 | 20 | 2003 | 23.5 | 2008 | - | - | - |
| Portugal | 25 | 34 | 2009 | 31.5 | 2008 | - | - | - |
| Romania | 19 | 19 | 2009 | - | 2008 | - | - | - |
| Slovenia | 19 | 27 | 2008 | 25 | 2004 | - | - | - |
| Slovakia | 18 | 20 | 2009 | 27 | 2008 | - | - | - |
| Finland | 17 | 16 | 2005 | 17 | 2008 | - | - | - |
| Sweden | 14 | 14 | 2003 | 13 | 2008 | - | - | - |
| United Kingdom | 29 | 27 | 2007 | 27.7 | 2008 | 22.5 | 81 | 2007 |

[13] AVEC (Association of Poultry Processors and Poultry trade in the EU Countries), 2009. Annual Report. Available at: http://www.thepoultrysite.com/articles/1531/poultry-meat-the-future-consumers-energy-and-the-environment

## E. PATHOGENESIS AND EPIDEMIOLOGY OF *SALMONELLA* IN BROILER MEAT PRODUCTION

### 1. Pathogenesis of *Salmonella* infection in broiler chickens

Young chicks of less than 1 week of age are susceptible to a broad range of non-typhoidal *Salmonella*, and the resulting infection is determined at least as much by the serovar as by other factors (infecting dose, age, genetic resistance, stress and predisposition of chicks). Besides discussing the above factors and their consequences, it should be noted that the possible differences between layer chicks and broiler chicks in pathogenetic processes, as a result of infection by the same strains and same dose of *Salmonella*, can not be described due to lack of exact comparative studies. Although it is thought that there can be apparent differences between susceptibility of different genetic lines of commercial hybrid birds, though not of the magnitude observed when unimproved native breeds are compared with substantially more susceptible commercial hybrids (Schou et al., 2009; Wigley, 2004) . There are a small number of in vitro studies indicating that the early innate immune response of heterophils to *S.* Enteritidis from laying hens (Leghorn) and broilers does not differ in a substantial way (Redmond et al., 2009; Swaggerty et al., 2006). The following section will aim to summarize knowledge regarding *Salmonella* infection of young chicks with special consideration of broiler age and conditions of broiler production.

As stated above, commercial broilers are young susceptible birds usually kept in high numbers and high density providing an ideal *in vivo* environment for almost any serovar of *Salmonella enterica.* The most striking difference in consequences of infection is between the freshly hatched chick (without intestinal flora) and the more mature birds. Newly hatched chicks can be successfully colonized and infected, sometimes with lethal outcome, with low doses of certain strains of *Salmonella,* and higher doses can result in clinical disease and mortality, while older birds are much less susceptible to colonization of their intestine and to the lethal effects of *Salmonella*e. Mortality due to experimental oral infection with *S*. Typhimurium can be up to 80% at one day of age and only 3% at 2 days of age (Smith and Tucker, 1980), although illness is rarely apparent in natural infections. The development of resistance by age to *Salmonella* has been primarily attributed to the intestinal microflora that produces antagonistic factors (i.e. short chain fatty acids), and/or competition for intestinal receptors (Gast and Beard, 1989). Pathogenetic outcomes of *Salmonella* infections in chicks follow in three main stages:

1. Intestinal colonization, which is normally the first consequence of oral infection, and usually leads to persistent shedding of the infecting strain of *Salmonella*.

2. This is almost inevitably followed by the invasion (or uptake) of *Salmonella* beyond the intestinal epithelial layer, and further into the lymphatics, and into the reticuloendothelial tissue, especially that of the spleen and the liver. The intensity and speed of the above pathogenetic processes correlate strongly with the oral infection dose (Bohez et al., 2006; Deng et al., 2008) and with serovar (Aabo et al., 2002; Berndt et al., 2009).

3. In cases of high infecting dose and/or high susceptibility, bacteriaemia may occur, occasionally causing varying degrees of mortality. In such cases *Salmonella* can be cultured not only from liver and spleen but also from the heart and bone marrow. Mortality due to naturally occurring *S.* Enteritidis and *S*. Typhimurium infection usually reaches peak levels at 3-7 days of age (Dhillon et al., 1999; Morris et al., 1969; Poppe et al., 1993). Non-invasive serovars (*S*. Kedogou, S. Heilderberg, S. Infantis, S.Hadar,) considered to be spread more horizontally than vertically, are expected colonize the intestine of young chicks more efficiently and are less invasive for internal organs (Barrow et al., 1988), which is also reflected in lower mortality as compared to *S.* Enteritidis and *S*. Typhimurium (Roy et al., 2001).

However, under normal husbandry conditions and management systems, such severe stages of *Salmonella* infection are quite rare. Instead, persistence of *Salmonella* is more characteristic in broilers (mainly in caecal tonsils), and results in a longer period of faecal shedding. Persistence and shedding

is also dependent on the age of birds at the time of infection. As an example, *S.* Typhimurium persisted for 7 weeks after oral infection more often when chicks were infected at 1 day of age compared with infection at 7 days of age (Gast and Beard, 1989). Similar age dependence has been observed for persistence and shedding of *S.* Enteritidis in young chicks (Gorham et al., 1991). The shedding pattern may also depend on the infective serovar or strain. Experimental infection of chickens with *S.* Hadar shows somewhat different shedding pattern than *S.* Typhimurium; the former may be shed for several weeks (Desmidt et al., 1998), while the latter is shed at high levels for only 1-2 weeks (Barrow et al., 1988).

Predisposing factors, such as immunosuppressive virus infection and coccidiosis, have been shown to increase the severity of *Salmonella* infection in poultry, and one of the mechanisms could be the decreased levels of caecal volatile fatty acids (Baba et al., 1985; Kubena et al., 2001; Qin et al., 1995). Exposure to infectious bursal disease virus may lead to increased mortality due to *Salmonella* infection, which may be explained by decreased B cell activity (Wyeth, 1975). Unfavourable environmental and management factors such as low brooding temperature, transport stress, water deprivation or changes in feed formulation or quality, may also decrease resistance of chicks to *Salmonella*, and consequently increase the incidence and severity of intestinal lesions and increase the level of faecal shedding (Thaxton et al., 1974). Use of antimicrobials may enhance colonisation as a result of perturbation of the normal competitive intestinal flora, especially in the case of *Salmonella* strains that are resistant to the antimicrobial that is used (Garner et al., 2009).

Commercial broilers are not normally vaccinated against *Salmonella* infection because of their short life span, low individual value and the requirement to avoid contamination of meat by live vaccine strains. Vaccination of broiler parents for *S.* Enteritidis, and in some cases *S.* Typhimurium, is however carried out in many countries. Such vaccination is likely to reduce vertical transmission of *Salmonella* from infected breeding flocks but may also provide some early protection of progeny, via maternal antibodies in yolk, when parenteral vaccines are used (Barman et al., 2005; Inoue et al., 2008). It is possible however that vaccination may have a sub-total effect, reducing the within flock prevalence and mean number of excreted organisms so that infected broiler breeder flocks may be more difficult to detect. Passive maternal immunity in commercial broiler birds is short lived so no adverse effect of detection at the normal monitoring age would be expected, although there have been only a few published studies on this aspect (Barrow, 2007; Kaiser et al., 2002; Zamora et al., 1999) Due to these limitations, there are no *Salmonella* vaccines on the market that would be specifically recommended for protection of broiler chicks at present.

## 2. *Salmonella* sources and risk factors related to the on farm production

Commercial broilers and other meat chickens are young susceptible birds that can readily become infected with *Salmonella* via a number of transmission routes (Davies, 2005; Mead, 2004). Quantitative risk assessment therefore offers a means to assess the relative contribution of control measures applied at various stages of production (Duffy, 2005).

### 2.1. Specific sources

#### *Vertical transmission from infected breeding flocks*

True vertical transmission occurs as a feature of *Salmonella* strains that are capable of colonisation or persistence in ovarian or oviducal tissue. This is often serovar related and *S.* Enteritidis is the major zoonotic serovar that has such abilities (EFSA, 2010d),along with certain strains of some other serovars, e.g. *S.* Typhimurium, *S.* Heidelberg, *S.* Infantis and *S.* Berta. Infected breeding flocks are therefore a major source of *S.* Enteritidis (Altekruse et al., 2006; Barbour et al., 1999; Davies et al., 1997; Feberwee et al., 2000; Kim et al., 2007; Liljebjelke et al., 2005) but a less frequent source of most other serovars (Bailey et al., 1999; Bailey et al., 2001; Brown et al., 1992; Byrd et al., 1999; Chriel et al., 1999). Control of *S.* Enteritidis by vaccination of breeding flocks for *S.* Enteritidis had a major effect on levels of that serovar found in broiler flocks (Feberwee et al., 2000; Snow et al., 2008).

***Pseudovertical transmission***

Pseudovertical transmission is associated with contamination of the hatchery environment or equipment in which eggs are processed and hatched or chicks held and handled. It is likely that the primary source of hatchery contamination is contaminated eggs, trays or trolleys originating from breeding flocks, but the source is unclear in many cases.

Spread of contamination within a hatchery is most dramatic when hatching eggs are infected with *S.* Enteritidis, in which case one infected breeding flock can lead to spread of contamination to multiple broiler flocks when chicks are hatched in the same premises. *S.* Enteritidis and *S.* Typhimurium do not appear to become resident in hatcheries, independent of infected eggs being processed, but other serovars, particularly those which are associated with contamination of feed processing premises and are able to survive well in the environment (Pedersen et al., 2008), may become established (Bailey et al., 1999; Christensen et al., 1997) . These strains can become persistent in biofilms within hatcher ventilation ducting, humidifying systems or door seals or may be continuously cycled from one hatch to another when there is pressure on time and space.

Another important source of recycling is inefficient crate washing, which results in contaminated hatcher baskets or egg trays being used for incubation. In some cases resident hatchery contamination is thought to have been introduced by washing contaminated crates from another company or by hatching eggs from sources other than the usual suppliers, including imported eggs, when there are shortages or economic advantages. The hatchery is therefore a critical control point in broiler production (Cox et al., 1991; Davies and Wray, 1994; Heyndrickx et al., 2007; Heyndrickx et al., 2002).

***Feed contamination***

Contamination of vegetable protein materials such as soya bean meal for poultry rations is relatively common (EFSA, 2008a) and this can lead to contamination of specific finished feeds if there is no effective decontamination stage (Jones et al., 1991). In some cases resident contamination may become established in compound feed mills, usually as a result of biofilm formation in coolers for heat treated pellets or meal rations after contaminated ingredients have been processed (Chadfield et al., 2001; Davies and Hinton, 2000; Davies and Wray, 1997a; Vestby et al., 2009).

*Salmonella* serovars originating from feed are frequently represented amongst infections in broiler flocks and are likely to cause early infections in chicks as a result of the immature intestinal flora and immune system of the birds during the first week of life (Beal et al., 2004; Holt et al., 1999; Rose et al., 1999), although, in general, the most common *Salmonella* serovars isolated from feed are rarely the most prevalent amongst those isolated in animals since the virulence of strains that are adapted to dry feed production conditions is likely to be low and consequently the infection may be transient, or the within-flock prevalence may be too low for detection by routine monitoring (Davies and Hinton, 2000; EFSA, 2007e)

Feed formulation may also influence the susceptibility of birds to intestinal colonisation as finely ground pelleted rations can induce a dysbacteriosis that interferes with competing bacteria(Bjerrum et al., 2005; Huang et al., 2006).

***Residual contamination in broiler houses***

It is relatively common for cleaning and disinfection of broiler houses to be incomplete and biofilm-forming serovars that have originated from feed may be more difficult to eliminate (Marin et al., 2009; Moretro et al., 2009). However, this does not always result in infection of flocks even though failed disinfection is highly predictive of a positive flock (Rose et al., 2003).

Certain *Salmonella* strains appear to be better able to persist than others, e.g. *S.* Paratyphi B var Java (i.e. *S.* Java), *S.* Infantis (Asai et al., 2007; Nogrady et al., 2008; van de Giessen et al., 2006). Since transmission of *Salmonella* among birds is dose-dependent (Byrd et al., 1998), factors such as stress levels and management factors may also be involved (Bessei, 2006; Humphrey, 2006; Jones et al., 2005). Free-range systems appear to be at reduced risk despite more difficulties with disinfection (Esteban et al., 2008; Tuyttens et al., 2008). Intercurrent disease such as coccidiosis can also increase the risk of colonisation by *Salmonella* (Koinarski et al., 2005).

Use of prophylactic antimicrobials may also play a part as increased or reduced risk has been reported in various studies (Cardinale et al., 2004; Evans and Wegener, 2003; Heyndrickx et al., 2007; Hughes et al., 2008; Rose et al., 2000) depending on the timing of treatment and resistance profile of the *Salmonella* strains concerned.

Wildlife vectors such as rodents that can amplify the numbers of *Salmonella* organisms and defecate directly into feeding systems can increase the likelihood of carry-over of infection between flocks, or spread of infection between flocks on a holding, especially if a multi-age production system is operated (Davies and Wray, 1995a; Meerburg and Kijlstra, 2007). If there is a high level of *Salmonella*, vector population and a short empty-time for houses between flocks passive carriers such as litter beetles may also become relevant (Crippen et al., 2009; Hald et al., 1998) but their involvement is highly variable (Davies and Wray, 1995b; Skov et al., 1999b; Wales et al., 2009).

### 2.3. Summary of risk factor analysis studies

Various cross-sectioned surveys or case control studies have been carried out to assess the prevalence of *Salmonella* on broiler farms and to identify potential risk factors or apparently protective factors.

The findings usually add support to those of descriptive studies addressing those factors representing an important risk for *Salmonella* contamination:

- contaminated feed (Angen et al., 1996; Henken et al., 1992),
- hatcheries (Angen et al., 1996; Chriel et al., 1999),
- infected breeding flocks (Maijala et al., 2005; Skov et al., 1999a),
- infection in previous flock in the house (Angen et al., 1996; Rose et al., 2000),
- poor biosecurity and deficiencies in cleaning and disinfection (Elgroud et al., 2009; Gradel and Rattenborg, 2003; Henken et al., 1992; Rose et al., 1999, 2000),
- quality of staff (Namata et al., 2009),
- medication (Chriel et al., 1999), or
- presence of rodents (Rose et al., 2000).

Paradoxically however free-range production often appears at reduced risk, but this may be partly associated with the greater age of birds at sampling (Snow et al., 2008). There is also evidence of a seasonal effect with higher levels of infection present in the Autumn (Angen et al., 1996; van der Fels-Klerx et al., 2008).

Other transport and management related factors are associated with a higher risk of carcase contamination after slaughter (Arsenault et al., 2007; Cardinale et al., 2005) and risk modelling approaches can be used to help evaluate the potential effect of control measures (Chriel et al., 1999; Maijala et al., 2005; Nauta et al., 2000; Oscar, 2004a). A major limitation of analytical approaches such as risk factor analysis is the degree of confounding which occurs that leads to failure to identify

many major known risk factors in multivariate analysis. One such important factor is the influence of integrated companies which have common farm designs, procedures and endogenous sources of infection within the company (Gutierrez et al., 2009; Heyndrickx et al., 2007; Liebana et al., 2002).

Confounding due to lack of key data and inclusion of data from diverse sources affected the analysis of the EU baseline survey for *Salmonella* in broiler flocks (EFSA, 2007e). It is therefore important to critically evaluate results of univariable analyses in the light of other studies and expert knowledge and to confirm the validity of apparent risk factors by controlled intervention studies.

### 2.5. Control measures to reduce *Salmonella* during broiler production

Improved monitoring and identification of persistent sources of infection in feedmills and hatcheries is essential so that control measures can be applied to such ongoing sources (Cadirci, 2009). It is often economically difficult to interrupt feed production or hatching for a long enough period to eliminate contamination but fortunately *S.* Enteritidis and *S.* Typhimurium (Vestby et al., 2009) do not become resident in hatcheries or feed coolers if there is no current source of contaminated eggs or feed ingredients.

It is not possible to fully control sporadic introduction of *Salmonella* under commercial conditions in countries with a large output of broilers but multifactorial control if properly applied can be cost-effective for society as a whole (Wegener et al., 2003) so a mechanism needs to be found to reward producers for such efforts although such good practices may also improve flock performances (Tablante et al., 2002).

Once flocks are found to be infected there are still some things that can be done before slaughter to reduce carcase contamination now that antibiotic treatment (Reynolds et al., 1997) is no longer allowed. Non-antibiotic interventions such as acidification of water and/or feed, competitive exclusion (CE) treatment or vaccination of parent flock can be of some use (Barrow et al., 2003; Van Immerseel et al., 2005). The use of CE can also reduce the weight of contamination that enters the slaughter process. However, *Salmonella* is most effectively controlled by elimination of infection from contaminated houses between flocks (Avila et al., 2003; Byrd et al., 2003).

### 2.6. Concluding remarks

- Feed, hatcheries and persistent contamination of holdings are the major sources of *Salmonella* in commercial broiler production.

- Each of these sources can be controlled, but in some cases control is difficult and costly.

- Apart from *S.* Enteritidis and *S.* Typhimurium, for which other major sources exist, there is little correlation between predominant *Salmonella* serovars that are found in broiler flocks and in humans in most MSs.

- International trade in contaminated broiler meat can, however, be responsible for spread of less common *Salmonella* serovars to the human population in importing countries (Threlfall al 2003) and international trade in hatching eggs can disseminate new strains of *Salmonella* to broiler production in importing countries. Such strains may later become established as residents in the importing countries unless they are contained by effective control measures.

- EU wide targets and other control measures for *S.* Enteritidis and *S.* Typhimurium should be continuously tightened as the prevalence in EU MS improves.

- The efficiency of detection of positive flocks should be investigated by comparing the results of operator and official testing

- A harmonised plan for introduction of additional specific control measures for other serovars should investigated for application in MSs if the incidence in humans is significant, or if there are serious issues relating to antimicrobial resistance or virulence

**References Appendix E**

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## F. AN INVENTORY OF RISK ASSESSMENT STUDIES

| Country | Author | Year | Aim of the risk assessment | Main findings |
|---|---|---|---|---|
| USA | Oscar | 1998 | To determine the relationship between the level of *Salmonella* contamination on chickens at the processing plant exit and the risk of salmonellosis for consumers. | Highly contaminated birds did not necessarily pose a greater risk of salmonellosis than lightly contaminated chickens. Greater risks were associated with temperature abuse, undercooking, and consumption by the high risk population. |
| The Netherlands | Nauta et al. | 2000 | To predict the effects of intervention strategies in the breeding pyramid for *Salmonella* control. | Based on expert opinion, sensitivity analysis indicated where most effective control measures could be implemented. The model was developed for the breeding pyramid of layers but may also be applicable for broilers. |
| Finland | Ranta et al. | 2002 | To describe the unknown true prevalences, vertical and horizontal transmissions, as well as the dynamical model of infections of *Salmonella* in the primary broiler production chain. | Eliminating *Salmonella* positive breeding flocks reduced the predicted flock prevalence from [1.3%-17.4%; 95% predictive interval] to [0.9%-5.8%]. In the scenario of one infected grandparent flock, these were [2.8%-43.1%] and [1.0%-5.9%], respectively |
| France | Rose et al. | 2003 | To assess the risk of contamination of chicken-broiler flocks by *Salmonella* at the end of the rearing period. | The risk estimated by the model was compared to the *Salmonella* status of the flock (gold standard) assessed by samples taken from the environment of the broilers and analysed with classical bacteriological methods. The sensitivity was 97.8% and the specificity 64.3%. |
| USA | Oscar | 2004 | To use published data and predictive models as input in a previously developed but updated model for *Salmonella* and whole chickens. | The incidence (prevalence!) of *Salmonella* contamination changed from 30% at retail to 0.16% after cooking to 4% at consumption. *Salmonella* growth on chickens during consumer transport was the only event that did not affect risk. Model predictions were similar to epidemiological data, despite numerous assumptions and simplifications. |
| UK | Parsons | 2005 | To identify and quantify all sources of contamination throughout the entire poultry meat production chain from the breeder farm to the chilled carcass by *Salmonella* spp. To compare three approaches to QMRA modelling: Bayesian networks (BN), Markov chain Monte Carlo (MCMC) and simulation (SIM). | All modelling approaches had advantages and disadvantages. BN propagates evidence from any point in the network to all others, but requires discrete variables. SIM is most flexible but may be more complex to implement and cannot propagate evidence. MCMC does not require discrete variables and is able to draw inference from evidence, and appears to be a good compromise. |

| Country | Author | Year | Aim of the risk assessment | Main findings |
|---|---|---|---|---|
| The Netherlands | Straver et al. | 2007 | To characterize the number of *Salmonella* on chicken breast filet at the retail level and to evaluate if this number affects the risk of human salmonellosis. | 8.6% of filets were contaminated above the detection limit of the MPN method (10 *Salmonella* per filet). Over two-thirds of annual predicted illnesses were caused by the small fraction of filets containing more than 3 log *Salmonella* at retail (0.8% of all filets). The enumeration results can be used to confirm this hypothesis in a more elaborate risk assessment. Modelling of the supply chain can provide insight for possible intervention strategies to reduce the incidence of rare, but extreme levels. Reduction seems feasible within current practices, because the retail market study indicated a significant difference between suppliers. |
| The Netherlands | Nauta et al. | 2005 | To describe the effects of inactivation and removal of bacteria from chicken carcasses and the dynamics of cross-contamination. The model was primarily developed for Campylobacter, but was suggested to be applicable to other bacteria as well. | The effects of inactivation and removal were found to be dominant for carcasses with high bacterial lads, and cross-contamination was dominant for those with low initial levels. Assuming a (log) linear relationship between input and output of processing stages was not realistic. The effects of logistic slaughter were predicted to be small. |
| Finland | Maijala et al. | 2005 | To study the public health effects of the Finnish *Salmonella* control program for broilers. To estimate the prevalence of contaminated meat from observed data. To estimate the effect of eliminating breeder flocks which have tested positive and of heat-treating meat of meat of detected positive broiler flocks. | Not removing positive breeder flocks would result in 1.0-2.5 (95% predictive interval) fold more human cases, no heat treatment would result in a 2.9-5.4 fold increase and without both interventions the increase would be 3.8-9.0 fold. Scenario analysis suggested that replacement of current meat (prevalence 0.21%) by meat with 20-40% contamination would increase consumer risk by 33-93 times. |
| Denmark | Christensen et al.(Evers and Chardon, 2010) | 2005 | To address individual hygiene practices during food preparation and consumption patterns in private homes. | The probability of ingesting a risk meal was highest for young males (aged 18-29 years) and lowest for the elderly above 60 years of age. This was ascribed to variations in the hygiene levels of food preparers. The model was primarily developed for Campylobacter, but may be applicable to other bacteria as well. |
| The Netherlands | Van der Fels-Klerx et al. | 2008 | To demonstrate how Performance Objectives (POs) for *Salmonella* at various points in the broiler supply chain can be estimated, starting from pre-set levels of the PO in finished products | Results indicated that, in general, decreasing *Salmonella* contamination between points in the chain is more effective in reducing the baseline PO than increasing the reduction of the pathogen, implying contamination should be prevented rather than treated. Application of both approaches at the same time showed to be most effective in reducing the end PO, especially at the abattoir and during processing. |

| Country | Author | Year | Aim of the risk assessment | Main findings |
|---|---|---|---|---|
| Belgium | Namata et al. | 2009 | To answer two questions:<br>(i) given the *Salmonella* status of the farm at a certain occasion (equal to the sampling time of the flock), what are the risk factors that the farm will be *Salmonella* positive at a following occasion?<br>(ii) what are the risk factors for a farm to be persistently positive for two consecutive flocks? | Many factors influence *Salmonella* risk in broiler flocks, and they interact. Accounting for that interaction leads to an improved determination of those risk factors that increase infection with *Salmonella*. A variety of risk factors are identified and the interactions between them that are accounted for described. |
| The Netherlands | Evers et al. | 2010 | To describe a simplified QMRA model especially aimed at comparing the risk of pathogen–food product combinations. | Results indicate that the sQMRA-tool is useful for quickly obtaining relative risk estimates of pathogen–food combinations. It can thus serve as a guide for selection of combinations for applying full-scale QMRA, or for risk management – by facilitating the translation of the results of trend analysis or of a specific research project into terms of risk. |

**References Appendix F**

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