European Food Safety Authority

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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 laying hens [1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Public health risks of *Salmonella* infection in laying hens (*Gallus gallus*) can be associated with exposure through four different pathways: internally contaminated table eggs, externally contaminated table eggs, egg products and meat from spent hens. In relation to eggs, *Salmonella* Enteritidis is by far the serovar most frequently associated with human illness, and exposure through eggs that are internally contaminated with this serovar has a higher public health significance than exposure to externally contaminated eggs. A mathematical model, using reported field data from two EU Member States (MSs), suggests a linear relationship between the investigated scenarios of flock prevalence for *Salmonella* Enteritidis and the number of contaminated eggs that would be laid. However, the absolute public health impact of the assessed flock prevalence scenarios is highly uncertain due to lack of data on the number of contaminated eggs produced by infected flocks and on the true number of egg-related human salmonellosis cases. It is suggested that public health benefits, similar to those obtained reaching lower *Salmonella* flock prevalences, may be achieved by implementing controls based on more sensitive sampling protocols. Diversion of eggs from flocks that are tested positive in the EU *Salmonella* control programme to the production of egg products subjected to heat treatment may lead to increased health risks as heat treatment of egg products should not be considered an absolute barrier to *Salmonella* contamination. Fresh meat from spent laying hens might carry a higher prevalence of *Salmonella* than meat from broiler flocks, in particular if sourced from *Salmonella*-positive flocks. The quantification of under-ascertainment and underreporting of human salmonellosis cases, improving knowledge on within-flock dynamics of *Salmonella* and harvesting data on production of *Salmonella* contaminated eggs under field conditions would contribute to improving the accuracy of future quantitative estimates.

KEY WORDS

Salmonella, microbiological target, laying hen, eggs, quantitative microbiological risk assessment.

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SUMMARY

Following a request from the European Commission, the Panel on Biological Hazards (BIOHAZ) was asked to deliver a Scientific Opinion on a quantitative estimate of the public health impact of setting a new target for the reduction of *Salmonella* in laying hens. In particular, EFSA was asked to assess the relative public health impact if a new target for reduction of *Salmonella* is set for laying hen flocks being 1% or less remaining positive for all *Salmonella* serovars with public health significance, compared to (1) a theoretical prevalence of 2% of flocks remaining positive for *Salmonella* Enteritidis or *Salmonella* Typhimurium, and (2) the real prevalence in 2008 to be reported by the Member States (MSs).

For this task, the BIOHAZ Panel took into account data from the first year of the harmonised EU-wide monitoring and control programme of *Salmonella* in laying hens plus sample level detailed data from two MSs. These data were used in the quantitative risk assessment modelling work on *Salmonella* in shell eggs that was coordinated by the EFSA's Assessment Methodology Unit (AMU) and which supports this Scientific Opinion of the BIOHAZ Panel.

The BIOHAZ Panel concluded that public health risks of *Salmonella* infection in laying hens are associated with four different exposure pathways: internally contaminated table eggs, externally contaminated table eggs, egg products and meat from spent hens. Taking into consideration factors such as time, resources and data limitations, the quantitative aspects of this Scientific Opinion focus on the calculation of the estimated number per million of shell eggs that are internally and/or externally contaminated with *Salmonella* Enteritidis, and that would be produced by certain scenarios of *Salmonella* flock prevalence. The risk assessment does not include the steps in the food chain after laying the eggs to consumption. There is considerable variation between and within EU MSs in many factors during handling, packaging, storage and preparation of shell eggs that may affect the risk of salmonellosis for which virtually no data are available. Furthermore, the request by the Commission does not include the evaluation of any additional interventions in these steps.

In the EU, two serovars (*Salmonella* Enteritidis and *Salmonella* Typhimurium) are considered of paramount public health significance. Together, they account for approximately 80% of all human isolates to which typing was applied. Other serovars do not individually exceed 1%.

Attribution models from two MSs and outbreak data from the EU suggest that, in relation to eggs from *Gallus gallus*, *Salmonella* Enteritidis is by far the serovar most frequently associated with human illness. This is related to the ability of this serovar to persistently colonise the avian reproductive tract, resulting in internally contaminated eggs, as well as egg shell contamination. Even though other serovars of *Salmonella* can be transmitted by egg shell contamination, the public health impact of this pathway is considered smaller compared to transmission by eggs internally contaminated with *Salmonella* Enteritidis. Therefore, the expected public health benefit of including additional serovars other than *Salmonella* Enteritidis in EU-wide prevalence targets for laying hens is expected to be small at present.

The quantitative risk assessment model used to support this Scientific Opinion suggests a linear relationship between the flock prevalence as currently observed in different MSs and the number per million of eggs contaminated with *Salmonella* Enteritidis. Based on the median estimates from the model, changing from the EU average flock prevalence reported in 2008 (3.1% for *Salmonella* Enteritidis) to a transitional EU target of 2% is expected to result in an approximately one third reduction in the number of *Salmonella* Enteritidis contaminated eggs produced the EU. Changing the EU target from 2% to 1% of flocks remaining positive would result in a further reduction of a similar order of magnitude in the number of contaminated eggs produced in the EU.

However, the absolute benefits of these reductions in flock prevalence are highly uncertain. There is a lack of data on the number of contaminated eggs produced by infected flocks, and on the true number

of egg-related cases of human salmonellosis. For two MSs, for which data in a suitable format for the model were available, there was a different prediction of egg prevalence at the same level of observed flock prevalence. This observation may be related to differences in production systems (e.g. housing, vaccination, hygiene practices), or the efficiency of detection of positive flocks.

The BIOHAZ Panel further concluded that the diversion of eggs from flocks that are tested positive to the production of egg products subjected to heat treatment may lead to increased health risks, as pasteurisation may not be an absolute barrier to *Salmonella* contamination. Monitoring data at the EU level suggest that in 2008 there was a higher proportion of samples of egg products that did not conform with EU food safety criteria when compared to the previous two years.

There are insufficient data to quantitatively evaluate the risk associated with human consumption of meat from spent hens when marketed as fresh meat. It is anticipated that the prevalence of *Salmonella* (including *Salmonella* Enteritidis) in the meat from these flocks might be higher than in meat from broiler flocks, in particular if sourced from *Salmonella*-positive laying hen flocks.

The BIOHAZ Panel makes a series of further recommendations on data needs and modelling considerations in order to reduce the uncertainty of possible future quantitative risk assessments in the same subject.

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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* serotypes in different poultry populations within the frame of Regulation (EC) No 2160/2003[4] on the control of zoonoses. As a transitional measure, a limited number of serotypes have been considered for reduction during the first three years of the control programme. Before the end of this period, a review of the serotypes should be considered.

As regards laying hens, Regulation (EC) No 1168/2006[5] sets a target for reduction being annual reduction until 2% or less flocks remaining positive for *Salmonella* Enteritidis or *Salmonella* Typhimurium during a transitional period until 1 February 2011. The Regulation also harmonises the monitoring in laying hens in all Member States since the beginning of 2008. 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 February 2011, a cost/benefit analysis should be carried out (See flowchart included 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

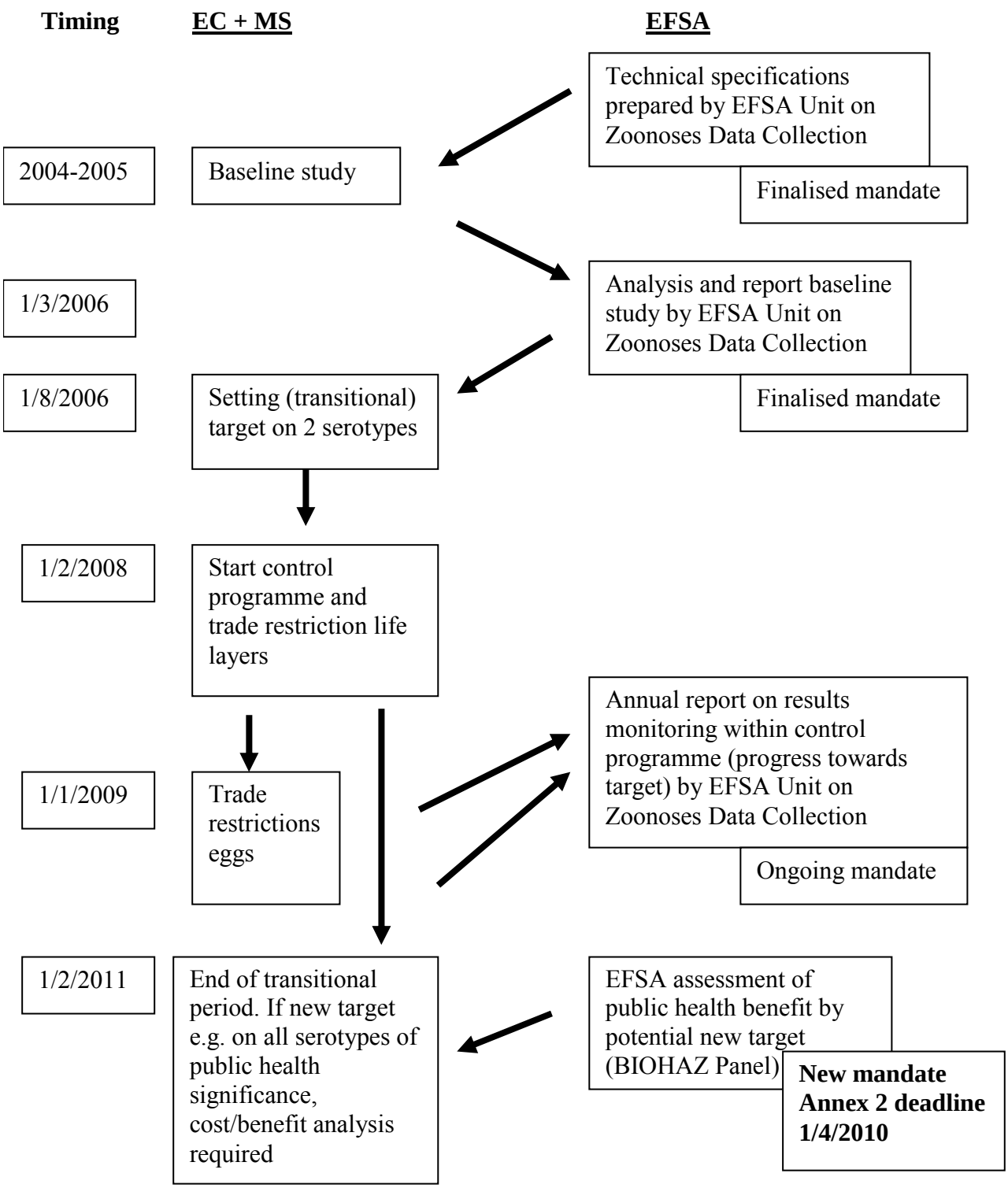
The EFSA is asked to assess the relative public health impact if a new target for reduction of *Salmonella* is set in laying hens being 1% or less remaining positive for all *Salmonella* serovars with public health significance, compared to:

- a theoretical prevalence of 2% of flocks remaining positive for *Salmonella* Enteritidis or *Salmonella* Typhimurium, and
- the real prevalence in 2008 to be reported by the Member States.

The *Salmonella* serotypes 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.

4 OJ L 325, 12.12.2003, p. 1. Regulation as last amended by Commission Regulation (EC) No 1237/2005 (OJ L 208, 24.10.2007, p. 5)
5 OJ L 211, 1.8.2006, p. 4.

Flowchart *Salmonella* control programmes laying hens and needs for EFSA input



ASSESSMENT

1. Introduction

This EFSA Scientific Opinion is the second of a series of quantitative estimates of the impact of setting new targets for the reduction of *Salmonella* in certain poultry populations (*Gallus gallus*, from now on referred to as laying hens throughout the document), which were requested by the European Commission in a mandate in April 2008. The first of them, adopted in March 2009, dealt with a request to provide a quantitative estimation of the impact of setting a new target for the reduction of *Salmonella* in breeding hens of *Gallus gallus* on the prevalence of *Salmonella* in production lines (EFSA, 2009).

In this Opinion, a quantitative estimate of the public health impact of setting a new target for the reduction of *Salmonella* in laying hens is considered. According to the Terms of Reference presented in the background as provided by the European Commission, EFSA is asked to provide an estimate on the public health impact of different flock prevalence values (theoretical vs. reported for the year 2008) of different *Salmonella* serovar groups (i.e. *S.* Enteritidis and *S.* Typhimurium vs. all *S.* serovars with public health significance) in laying hens.

Field data for the calculation of these estimates will be sourced from the results of the first year of the harmonised EU wide monitoring and control programme of *Salmonella* in laying hens plus sample level detailed data from two MSs. EFSA wishes to acknowledge those MSs that submitted sample level detailed data for its consideration. When feasible, these data were used in the quantitative risk assessment modelling work on *Salmonella* in shell eggs that was coordinated by the EFSA's Assessment Methodology Unit and which supports this EFSA Scientific Opinion of the Panel on Biological Hazards (Scientific Report of EFSA on a Quantitative risk assessment of *Salmonella* Enteritidis in shell eggs in Europe. EFSA Journal 2010; 8(4):1588, available at: www.efsa.europa.eu/en/scdocs/scdoc/1588.htm . Cited in this Opinion as EFSA, 2010a).

The three main food categories derived from the laying hen poultry industry linked to human salmonellosis that will be considered in this Scientific Opinion, are:

- Table eggs for direct human consumption;
- Pasteurised egg products for human consumption;
- Meat and meat products derived from spent laying hen flocks slaughtered for human consumption.

Taking into consideration factors such as time, resources and data limitations, the quantitative aspects of this Scientific Opinion focus on the calculation of the estimated number per million of shell eggs that are internally and/or externally contaminated with *S.* Enteritidis that would be produced by certain scenarios of *Salmonella* flock prevalence. The reasoning for focusing on *S.* Enteritidis in the quantitative assessment is developed throughout the document. The comparison of those scenarios (e.g. target prevalences against reported prevalences) should provide an indirect estimate of the public health impact of different *Salmonella* flock prevalences. The risk assessment does not include the steps in the food chain after laying the eggs to consumption. There is considerable variation between and within EU MSs in many factors during handling, packaging, storage and preparation of shell eggs that may affect the risk of salmonellosis for which virtually no data are available. Furthermore, the request by the Commission does not include the evaluation of any additional interventions is these steps, and a proportional relationship between public health risks and *Salmonella* egg prevalence is expected (World Health Organisation and Food and Agriculture Organisation, 2002; EELA, 2006).

2. Public health significance of *Salmonella* in eggs and egg products

2.1. Reported and true incidence of salmonellosis in the EU

Details on the reporting system for human salmonellosis in the EU and the results until 2007 can be found in the previous EFSA Opinion related to targets for breeding hens (EFSA, 2009) and in the Community Summary Report (EFSA, 2010b). These documents also describe issues related to underreporting of human salmonellosis and indicates that the true burden may be considerably larger than the reported incidence, albeit that the level of underreporting varies strongly between MSs. "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.

Further evidence on underreporting comes from serosurveillance studies like the one completed as part of the Med-Vet-Net project (Falkenhorst and Simonsen, 2009) in which population representative serum samples of 8 MSs were analysed for antibodies against *Salmonella.* Subsequently, statistical models were used to estimate the incidence of new infections from the seroprevalence. It has to be noted that infection as measured by seroconversion does not necessarily imply clinical illness. No relation was found between the sero-incidence of *Salmonella* and reported laboratory findings of the contributing MSs, confirming the notion of large differences in sensitivity of the surveillance systems. Sero-incidence exceeded incidence of laboratory reported cases by a factor of 124 to ~10,000, depending on MS. However, the sero-incidence appears to be lower in MSs that have implemented control programmes for salmonellosis for a long time (Finland, Sweden, Denmark and the Netherlands). There is a correlation with country specific incidences derived from analysing data on travellers returning to Sweden. No serovar specific conclusions can be drawn as the mixed-Elisa used detects both the O-antigens of *S.* Enteritidis and *S.* Typhimurium.

At the EU-level, the under-ascertainment ratio of clinical illness is expected to range between 5 and 100 in different MSs. This would imply that in the EU27 (population on 1st January 2008 was around 500 million), the approximately 130,000 verified of human salmonellosis cases would translate into not less than 1 million and possibly as high as 15 million cases of clinical salmonellosis per year. A similar range of not less than 1 up to 6 million cases per year is suggested by the serosurveillance studies (see calculations in Appendix A).

In the Netherlands, *Campylobacter*, *Toxoplasma* and *Salmonella* are the three foodborne pathogens causing the largest disease burden[6]. The largest contribution to the burden is from complications. Mortality also causes a relatively high burden, while the contribution of acute gastroenteritis is relatively low. The burden of salmonellosis, including complications (Post-Infectious Irritable Bowl Syndrome (PI-IBS), reactive arthritis) in the Netherlands (43,000 cases per year, 50 fatal cases) is estimated at 1,600 DALYs[7] per year (Haagsma et al., 2010). The costs of salmonellosis and sequels (excluding post-infectious irritable bowel syndrome) in the Netherlands were estimated at 11 million € per year (Haagsma et al., 2009). Extrapolating these estimates would result in a disease burden of 0.02-0.5 million DALYs per year for the EU27 and total costs between 0.2 and 3 billion € per year.

The establishment of harmonised active surveillance of human salmonellosis in all MSs, including efforts to quantify the level of under-ascertainment and underreporting, would improve the evaluation of the human health effects of interventions in flocks of laying hens.

6 For further information see www.rivm.nl/vtv/object_class/kom_voedsel_micro.html

7 DALYs=Disability Adjusted Life Years. For further details see Murray CJL and Lopez AD (Eds). The global burden of disease: a comprehensive assessment of mortality and disability from disease, injury, and risk factors in 1990 and projected to 2020. Global burden of disease and injury series, volume I. WHO/Harvard University Press, 1996.

2.2. Attributing eggs and egg products to human salmonellosis

The relative frequency of serovars found in different animal populations differs from that in humans and dynamic changes occur between regions and animal populations and production types. However, there is overwhelming evidence that human infections with *S.* Enteritidis predominantly originates from eggs and egg products, while *S.* Typhimurium predominantly originates from cattle, pigs and poultry in different proportions (EFSA, 2010b). Such strains may have special 'fitness' potential in terms of virulence, dissemination or survival in the preferred host (Humphrey, 2004) or be associated with those hosts by means of trade in carrier animals (Witte, 2004).

Salmonella control programmes in laying hens (including eradication and/or vaccination) have been very effective in reducing the prevalence of human salmonellosis due to *S.* Enteritidis. In Denmark, an eradication plan for *Salmonella* in table-egg production was implemented in 1996. The proportion of infected layer flocks was reduced from 13.4% in 1998 to 0.4% in 2006 and in parallel the number of reported cases of human salmonellosis that was estimated to be associated with eggs decreased from 3,000 in 1997 (55-65% of all cases) to approximately 100 in 2006 (5-7% of all cases) (Korsgaard et al., 2009). After vaccination in 2004 in Belgium, laboratory confirmed cases of human salmonellosis dropped from 12,894 in 2003 (89% due to *S.* Enteritidis) to 3,831 in 2008 (Collard, 2008; EFSA, 2010b). In Austria, where from 2000 vaccination was advocated, salmonellosis dropped from 8,405 in 2002 (91% *S.* Enteritidis) to 3,196 in 2008 (Kornschober, 2009).

Such observations confirm that layers are a major reservoir of human salmonellosis and that infections in humans can be reduced considerably by preventing and controlling *S.* Enteritidis infections in laying hen flocks. *Salmonella* control programmes as used in different MSs have been accompanied by a concurrent decline in the incidence of human Salmonellosis, in particular due to *S.* Enteritidis, both in outbreaks and sporadic cases. Control programmes may also include restrictions on the shelf life of table eggs, which are believed to have also contributed to the decline in human salmonellosis in some countries.

In addition, two MSs, Czech Republic and Germany, reported a marked drop in the notification of human salmonellosis in 2008 compared to 2007 (41.8% and 33.9%, respectively). A drop in the number of human cases was also observed in Poland and the UK. The result from the monitoring programme for *Salmonella* in the layer flocks in 2008 in these four MSs showed that the results are way below their target (see section Table 6 in section 4.2).

The summer peak of *Salmonella* infections in humans is absent in broilers and layers. This points to the characteristic infection risk due to the combination of several factors: contaminated eggs that end up in foods, the growth in foods at ambient temperatures, the use of eggs in dishes that are not or only slightly heat treated and the ability of particularly *S.* Enteritidis to cause internal egg contamination. In Europe, seasonality is small for *S.* Typhimurium (and other non *S.* Enteritidis serovars): in 2006, 2007 and 2008 about 1.6 times higher numbers of cases appeared in the summer than in spring, whilst for *S.* Enteritidis this was up to 3.4 times higher in the summer (EFSA, 2007; EFSA, 2009a; EFSA, 2010b). Kovats and colleagues showed in a study published in 2004 that in 6-17 years of surveillance data from 5 MSs the risk of a *Salmonella* infection increases above a threshold of 6^0C with 9-12% with each increase of 1^0C. This empirical finding holds in particular for *S.* Enteritidis and to a much lesser extent for *S.* Typhimurium (Kovats et al., 2004) and is not considered a property of the serovars themselves but of their predominant food vehicles and the way these are treated before consumption. Recent publications of case-control studies of sporadic infections with *S.* Enteritidis, not *S.* Typhimurium, point to eggs and egg products as the predominant risk factor (Mølbak and Neimann, 2002; Doorduyn et al., 2006).

In Australia, *S.* Enteritidis is not endemic in the egg laying flocks presumably due to a different breeding stock (great grand and grand parents) population, and sporadic *S.* Enteritidis infections are almost all travel related (OzFoodNet, 2007). Furthermore, both *Salmonella* and egg-related outbreaks are less predominant than in Europe and most often implicate *S.* Typhimurium. Indeed Australian

investigators relate these outbreaks to eggshell contamination, i.e. soiled eggshells. The same applies to a recent overview comparing the EU, Australia, New Zealand and the US (Greig and Ravel, 2009). In areas where the number of egg-related *S.* Enteritidis outbreaks is low, the occurrence of egg-related *Salmonella* outbreaks is in general lower and no dominating serovar seems to be implicated. This indicates that the role of serovars other than *S.* Enteritidis, in most circumstances, is caused by faeces contaminated/soiled eggshells.

a. Attribution to commodities using outbreak data

A simple descriptive analysis or summary of outbreak data is useful for attributing illnesses to foods, but often the implicated food in an individual outbreak is a "complex" food, containing several food items. Any of these foods could be the actual source of the infection. Focusing on the final vehicle of exposure, i.e. the food item consumed, may also give an incorrect picture since cross-contamination between different sources or food items may occur. Even in verified outbreaks, the original source of infection may not have been identified.

Several methods have been developed to use the information from outbreaks to attribute human illness to sources (e.g. Adak *et al.*, 2005; Greig and Ravel, 2009). However, common to them all are that they exclude information from outbreaks caused by complex food. 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 (Painter *et al.*, 2009). Foods that contain ingredients that are members of a single category are considered 'simple foods', while foods that contain ingredients that are members of multiple categories are considered 'complex foods'. As an example, steak is a simple food, whereas meat loaf is a complex food. For outbreaks that have implicated a simple food item, all illnesses are attributed to a single category. For outbreaks that have implicated a complex food item, illnesses are partitioned to all the categories making up the complex food according to the proportion of illnesses attributed to these categories in outbreaks caused by simple foods. The number of illnesses attributed to each category is then summed to determine the percentage of disease attributed to each category.

This method has recently been applied by Pires et al. (2009) on *Salmonella* outbreaks reported to EFSA in 2005 and 2006. In these data no distinction between verified[8] and non-verified outbreaks was possible and the results should therefore be interpreted with care. Of 15 categories (dairy, poultry, chicken, turkey, beef, pork, lamb, game, fruits/nuts, vegetables, grains or beans, oils or sugar, seafood, and meat unspecified[9]), by far the largest proportion of outbreak-associated salmonellosis cases (~32%) were attributed to eggs, followed by meat including poultry (~ 11%) (see Table 1). However, a large group of "unknown" (about 42%) was not attributable to any of the discerned categories. Among illnesses attributed to a known source only, 58% of cases were attributed to the consumption of eggs, 20% to meat and poultry products and 7% to international travel. Attribution estimates differed between European regions[10]. Eggs were the most important source of outbreak-associated salmonellosis in Eastern (~61%) and Western Europe (~27%), but had in the latter twice as much "unknowns" as in the other regions (~56%). Chicken was the most important source in Northern Europe (~24%), followed by eggs (~21%); meat (unspecified) was the most important source in Southern Europe (~39%), also followed by eggs (~23%). The vast majority of the salmonellosis outbreaks that reported to have resulted in death were attributed to eggs (~67%). Surprisingly, a relatively large proportion of *S.* Typhimurium cases were attributed to eggs, but this proportion

8 An outbreak is considered *verified* if descriptive epidemiological evidence and either analytical epidemiological or laboratory evidence are available (Report of the Task Force on Zoonoses Data Collection on harmonising the reporting of food-borne outbreaks through the Community reporting system in accordance with Directive 2003/99/EC, *The EFSA Journal* (2007) 123, 1-16).

9 For many outbreaks "meat" was reported as the source without further specification (EFSA, 2010b).

10 European regions defined according to UN (Pires et al., 2009).

differed substantially between European regions and may reflect a true difference or an incorrect reporting of eggs as the causative source of outbreaks.

Table 1: Attribution estimates showing the proportion of outbreak-associated salmonellosis cases attributed to specific sources[1] in Europe, 2005 and 2006 (median, %).

	Proportion of number of ill	95% CI	Proportion of number of outbreaks	95% CI
Eggs	32.45	[20.89, 47.00]	25.72	[15.95, 40.33]
Meat and poultry, unspecified	11.10	[4.12, 22.07]	9.47	[4.08, 20.83]
Chicken	1.83	[1.05, 3.10]	1.60	[0.95, 2.67]
Dairy	2.21	[0.89, 4.46]	1.78	[1.09, 2.89]
Vegetables	1.39	[0.48, 2.85]	0.49	[0.24, 0.92]
Travel	3.89	[0.21,12.65]	2.16	[0.54, 6.45]
Unknown	42.02	[24.42, 59.33]	54,92	[31.86, 71.42]

1) Other sources (poultry, turkey, beef, pork, lamb, game, fruits and nuts, oils and sugar, seafood) were each estimated to contribute <1% of all outbreaks as well as all cases.

Pires and colleagues (2009) concluded that the results of the analysis of outbreak data were found useful to investigate the relative importance of food sources for human salmonellosis at the European level. It was, however, acknowledged that extrapolation from outbreak data to the population level involves making certain assumptions that may not reflect reality. Comparing and combining outbreak data from different countries is difficult due to lack of harmonisation of food categories, various reporting schemes and level of evidence even within countries. Investigators may be biased towards collecting data on the types of food perceived as high risk or laboratory methods may vary according to food type. Furthermore, certain food sources may be more likely to cause outbreaks than others and a proportion of sporadic cases may in fact belong to undetected outbreaks, altogether biasing the congruence of the relative contribution of each food type among outbreaks and among sporadic cases.

The EFSA Community Summary Report on Trends of Zoonoses and Zoonotic Agents for 2008 shows the predominance of the involvement of *Salmonella* in reported human foodborne outbreaks in Europe (Table 2). Between 2005 and 2008, the overall number of reported foodborne outbreaks did not vary much between the years. The fraction of outbreaks related to *Salmonella* decreased however from 64% and 55% in 2005 and 2006, respectively, to 35% in 2008 (Table 3). Faced with the cumbersome task of collecting validated data from all MSs, EFSA, in close collaboration with ECDC, implemented a new reporting system for foodborne outbreaks in 2007. The system guides and encourages MSs to collect harmonised epidemiological and laboratory data from foodborne outbreaks in order to allow for detailed attribution analyses. However, in 2007 some MSs had difficulties in complying with the guidelines and so national adjustments for the collection of data were implemented in 2008. This resulted in outbreak data of improved quality but also in a marked drop in the number of verified outbreaks. Due to the implementation of the new reporting system for foodborne outbreaks in 2007, it is not possible to draw direct comparisons of pathogens and sources, or conclusions between the reporting rates in 2005-2006 and 2007-2008.

Table 2: Number of reported food-borne outbreaks and human cases of salmonellosis, 2005-2008 (EFSA, 2006; EFSA, 2007; EFSA, 2009c; EFSA 2010).

	Reported food-borne outbreaks			Salmonellosis cases involved		
Year	**All agents**	***Salmonella***	**%**	**Cases**	**Hospitalized**	**Deaths**
2005	5,355	3,406	64	25,760	3,554	16
2006	5,705	3,131	55	22,705	3,185	23
2007*	5,609	2,201	39	9,062	1,785	10
2008*	5,332	1,888	35	14,180	2,868	20

* New reporting system implemented (Report of the Task Force on Zoonoses Data Collection on harmonising the reporting of food-borne outbreaks through the Community reporting system in accordance with Directive 2003/99/EC, *The EFSA Journal* (2007) 123, 1-16)

In verified foodborne *Salmonella* outbreaks, where the implicated food source was noted as "known", eggs or egg products were reported in 40.8% of the outbreaks in 2008. In addition, bakery products, which to a large extent probably also were caused by the use of contaminated eggs, were reported in 13.5% of the outbreaks. Among verified outbreaks in 2008 implicating eggs or egg-related products *S.* Enteritidis dominated (77.2%). Only few outbreaks involved *S.* Typhimurium in eggs and egg products (3.5%). In addition, 91.4% of all outbreaks caused by bakery products reported *S.* Enteritidis as the causative agent.

In a former BIOHAZ Scientific Opinion (EFSA, 2009d) a literature review indicated that in outbreaks occurring in the EU linked to the consumption of eggs or egg products, 97% involved *S.* Enteritidis as the causative agent and 2% *S.* Typhimurium. Nevertheless, caution was taken when interpreting reported outbreaks in literature due to the different level of ascertainment and the possibility for one outbreak to have been reported in more than one scientific paper.

b. Attribution using microbial subtyping

Whereas attribution using outbreak data generally takes place at the point of consumption, the microbial subtyping approach attribute human illnesses to the reservoir level i.e. the approach quantifies the contribution to human salmonellosis of the most important reservoirs. The method requires a heterogeneous distribution of *Salmonella* subtypes among the different reservoirs and is facilitated by a systematic surveillance of all important reservoirs providing a representative collection of isolates (Hald et al., 2004).

Source attribution information broken down on serovars associated with *Gallus gallus* is available only in a few MSs. Data based on microbial subtyping (see EFSA 2008b – *Salmonella* source attribution, for details) from the Netherlands and Denmark are presented in Table 3. This table shows that also in relation to layers/eggs, *S.* Enteritidis and, to a lesser extent, *S.* Typhimurium are estimated to be associated with the majority of human cases.

Table 3: Proportion of sporadic national cases (i.e. excluding outbreak and travel related cases) and serovar distribution of human salmonellosis attributed to *Gallus gallus* based on microbial subtyping between 2003 and 2008 in The Netherlands and Denmark (Raw data supplied by Wilfrid van Pelt (RIVM, Bilthoven, The Netherlands) and Tine Hald (FOOD-DTU, Soborg, Denmark).

Country	**The Netherlands 2003-2008**			**Denmark 2003-2008**			
Reservoir/vector	All endemic sources	Broilers, broiler meat	Layers	All endemic sources	Broilers National	Broiler meat, imported	Layers
Attributable fraction (all serovars)		9.5%	24.7%		2.5%	9.3%	8.9%
S. Enteritidis	41.7%	4.8%	20.5%	32.0%	0.1%	4.3%	8.0%
S. Typhimurium	36.0%	1.4%	1.5%	29.0%	0.7%	0.5%	0.4%
S. Hadar		0.2%	0.1%		0.0%	0.2%	0.0%
S. Infantis		0.5%	0.3%		0.2%	0.4%	0.1%
S. Virchow		0.2%	0.2%		0.1%	0.8%	0.0%
Other serovars		2.4%	2.1%		1.4%	3.1.%	0.4%

In the EU in 2008, 58% of all cases of human salmonellosis were associated with *S.* Enteritidis. A large share of these is due to eggs, although its exact contribution is unknown. A rough estimate based on an extrapolation from the attribution estimates presented in table 3 for The Netherlands and Denmark suggests that this proportion could be about 2/3, meaning that about 40% of all human salmonellosis in the EU might be associated with the consumption of contaminated eggs. Between MSs, however, the proportion of egg-borne infections will vary considerably depending on the *Salmonella* prevalence in layer flocks, the amount and origin of imported eggs, the amount of eggs consumed, and egg preparation and consumption habits.

The subtype-based attribution model initially described for Danish data was extended in 2007 (Hald et al., 2007) to include information on the antimicrobial susceptibility testing of *Salmonella* isolates from humans and sources. The results indicated that multi-drug and quinolone resistance was rarely found in cases acquired from Danish food, but was common in cases related to imported products (49.7% and 35.6% of attributable cases) and travelling (26.5% and 38.3% of attributable cases). For food consumed in Denmark, imported poultry meat and Danish eggs were found to be the most important source of quinolone-resistant strains (primarily *S.* Enteritidis), whereas multidrug-resistant strains (primarily *S.* Typhimurium) appeared to be associated with Danish and imported pork and imported beef.

c. Case-control studies

Case-control studies of sporadic infections are the most commonly applied approach for identifying possible exposures for sporadic foodborne disease. Selected case-patients (normally confirmed by culture) and a representative group of asymptomatic individuals (controls) are interviewed, and the relative role of exposures is estimated by comparing the frequency of exposures among cases and controls. When infections are associated with an exposure, the proportion of cases attributed to the exposure can be calculated and this measure is defined as the "population attributable fraction" (PAF) (Clayton and Hills, 1993).

Limitations of case-control studies include misclassification of exposures due to lack of accuracy of recall, which may lead to an underestimation of the burden of illness attributed to specific exposures. Likewise, misclassification due to immunity may reduce the attributable risk or even suggest protection. In many studies, food exposures only explain a small fraction of all cases, and cases may reflect a mixture of possible sources of exposure, which can make it difficult to distinguish between

these exposures. Lastly, statistical power to determine the importance of common exposures often requires enrolment of many participants and therefore a lengthy study period.

Numerous case-control studies of sporadic *Salmonella* infections have been published (Kapperud et al., 1998; Mølbak and Neimann, 2002; Kimura et al., 2004; Doordoyn et al.; 2006; Jones et al.; 2006; Marcus et al., 2007) and the results supports the conclusions that the consumption of raw or undercooked eggs is a major risk factor for human *S.* Enteritidis infections. Other significant risk factors included travelling abroad and eating chicken. For *S.* Typhimurium, consumption of meat was more often identified as a risk factor. Still, it should be noted that direct comparison of results between individual case-control studies often are difficult because authors have included different (groups of) exposures or defined the risk factors in different ways.

A systematic review of published case-control studies of sporadic infections of a given pathogen can provide an overview of the relevant exposures and risk factors for that infection, and a summary of the estimated population attributable fractions for each exposure. An overall population attributable fraction derived from a meta-analysis or weighted summary of several case-control studies of a certain pathogen can be combined with estimates of the burden of disease caused by that pathogen to estimate the burden of disease attributed to each exposure.

A systematic review of case-control studies was recently performed by Domingues et al. (2009) to identify risk factors for sporadic salmonellosis. A total of 34 papers were included from a variety of countries worldwide and published between 1989 and 2003. Two studies investigated risk factors in children, and three focused only on individuals above 10 years of age. Results from the overall meta-analysis, encompassing all age-groups, serovars and time periods, pointed at undercooked chicken as the most important food exposure, followed by consumption of raw eggs and beef. The confidence interval around the estimate for eating chicken undercooked was wide, and thus results should be interpreted with care. Results from a separate analysis for *S.* Enteritidis was highly influenced by US studies, and suggested that travel outside the US was a very important risk factor for infection with *S.* Enteritidis (for the American population), and that eating in a restaurant, eating home-made ice cream, chicken at a restaurant and undercooked and raw eggs followed in importance. Among direct contact with animals-routes, daily contact with a pet was a significant risk factor for *S.* Enteritidis infections.

It is likely that control of *S.* Enteritidis in laying flocks will also lead to reduced environmental contamination associated with the spreading of manure and wash water from contaminated laying houses. This should also favourably influence the potential for spread of contamination to other farm livestock species, companion animals and edible crops.

2.3. Regulatory criteria on *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 (EFSA, 2004 and 2009), and thus should be considered of public health significance. Nevertheless, there are differences between serovars in relation to their frequency in human illness and association with particular food chains that may affect food safety decision making. The relative importance of serovars originating from the poultry reservoir differs and dynamic changes occur between regions and production type. From a regulatory perspective, Annex III to Regulation (EC) 2160/2003 on the control of *Salmonella* and other specified foodborne zoonotic agents prescribes the specific criteria to determine *Salmonella* serovars with public health significance to which Community targets will apply. The criteria to be taken into account are as follows:

- the most frequent *Salmonella* serovars in human salmonellosis on the basis of data collected through the 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 animals;
- whether any serovars show increased virulence, for instance as regards invasiveness, or resistance to relevant therapies for human infections.

Current information on these criteria in relation to layers and eggs of *Gallus gallus* is presented in this document. Nevertheless, consideration has been given to those aspects in an earlier EFSA Opinion (EFSA, 2009) in the context of breeding hen production, which should still be a valid reference point.

a. Reported *Salmonella* serovars in human salmonellosis

Of the more than 2,500 serovars of *Salmonella*, *S.* Enteritidis has emerged over the past 15-20 years as the leading cause of human salmonellosis in many countries. A wide range of foods has been implicated in foodborne illness due to *S.* Enteritidis. . The *S.* Enteritidis pandemic which began in the mid-1980s has been widely attributed to increased foodborne illnesses associated with poultry and in particular outbreaks and sporadic cases associated with hens' eggs and egg products (Anon 2001, Cogan & Humphrey 2003; Lake *et al.* 2004; Voetsch *et al.* 2004).

The emergence of *S.* Enteritidis in table-egg layers and humans has been explained by the combination of two main factors: the extraordinary epidemiology of recently emerged strains of *S.* Enteritidis infections in laying hens due to vertical transmission of the infections and the centralised rearing of breeding stock spreading the infection to many parts of the world (Thorns, 2000). Other serovars have been less associated with egg contamination and egg-transmitted disease, but foodborne outbreak reports have attributed egg-transmitted disease to serovars such as *S.* Heidelberg, *S.* Typhimurium, and *S.* Infantis (e.g. Mitchell et al. 1989; Aseffa et al, 1994; Arnedo et al, 1998; Mason et al., 2001; Hall, 2002; Unicomb et al., 2003; CDC, 2004; Chittick et al., 2006; Greig and Ravel, 2009).

The ranking of the serovars most frequently isolated from cases of human salmonellosis in European countries for 2008, 2007 and 2006, as reported in the CSR, is presented in Table 4 (EFSA, 2009a; EFSA, 2010b). The review of these data may also indicate whether any serovar shows a rapid and recent ability to spread and cause disease in humans.

Table 4: Distribution of the 10 most frequent *Salmonella* serovars from laboratory confirmed salmonellosis cases in humans. TESSy data, 2006 – 2008 (EFSA, 2009a; EFSA, 2010b).

2008			2007			2006		
Serovar	**N**	**%**	**Serovar**	**N**	**%**	**Serovar**	**N**	**%**
Enteritidis	70,091	58.0	Enteritidis	81,472	64.5	Enteritidis	90,362	71.0
Typhimurium	26,423	21.9	Typhimurium	20,781	16.5	Typhimurium	18,685	14.7
Infantis	1,317	1.1	Infantis	1,310	1.0	Infantis	1,246	1.0
Virchow	860	0.7	Virchow	1,068	0.8	Virchow	1,056	0.8
Newport	787	0.7	Newport	733	0.6	Newport	730	0.6
Agona	636	0.5	Stanley	589	0.5	Hadar	713	0.6
Derby	624	0.5	Hadar	479	0.4	Stanley	522	0.4
Stanley	529	0.4	Derby	469	0.4	Derby	477	0.4
Bovismorbificans	501	0.4	Kentucky	431	0.3	Agona	367	0.3
Kentucky	497	0.4	Agona	387	0.3	Kentucky	357	0.3
Other	18,495	15.3	Other	18,562	14.7	Other	12,790	10.0
Total	**120,760**	100%	**Total**	**126,281**	100%	**Total**	**127,305**	100%
Unknown	6,636		Unknown	9,814		Unknown	17,359	

Reporting countries: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Luxembourg, Malta, the Netherlands, Portugal, S lovakia, Slovenia, Spain, Sweden and the United Kingdom (England, Wales, Scotland and Northern Ireland).

The data in Table 4 show that in the EU Total in 2006-2008, *S.* Enteritidis and *S.* Typhimurium were associated with about 80% of all reported and laboratory confirmed cases of human salmonellosis. 20% of cases were associated with a variety of other serovars, each contributing around or below 1%. 5-7% of *Salmonella* isolates were "unknown", which includes untyped isolates (no typing was attempted) and untypeable isolates (typing was attempted but outcome was not successful). The proportion of unknown has been decreasing from 13.6% in 2006 to 5.5 in 2008.

As seen in Table 4, the reduction in the overall numbers of reported cases of salmonellosis is caused by a reduction of *S.* Enteritidis infections. *S.* Enteritidis notifications decreased by 14% (4.8 cases per 100,000 population) in 2008 compared to 2007 and a total of 16 MSs reported fewer human *S.* Enteritidis cases. This is believed to be a true decrease since the notification rate for *S.* Typhimurium increased in the same time period and the number of cases reported as 'unknown' serovar decreased.

b. *Salmonella* serovars in laying hen production and eggs and egg products

Current regulatory requirements regarding reporting of *Salmonella* infected flocks of laying hens in the context or target monitoring is limited to *S.* Enteritidis and *S.* Typhimurium (Regulation (EC) No. 1168/2006). On the other hand, Regulation (EC) No. 2160/2003 requires that the serovars to be reported are those with public health significance, to be determined with the same guidance as the one followed in this section.

Overall and for laying hen flocks and due to the legal bases for reporting serovars, data on the occurrence of serovars other than *S.* Enteritidis and *S.* Typhimurium is not available for most MSs. Table 6 in section 4.2. shows that for the EU Total an average of 5.9% of the flocks were reported as positive to any *Salmonella* serovar during the production phase, out of which 52.5% were identified as *S.* Enteritidis, 8.5% as *S.* Typhimurium and 39% composed the group of other serovars, non-typeable and unspecified. These figures seem to be consistent with reported values from previous years, where *S.* Enteritidis accounted for more than 50% of the total *Salmonella* cases reported in laying hen flocks during production (see Appendix B).

In 2008, fifteen MSs reported data from investigations of table eggs (EFSA, 2010b). In total, 0.5% of the tested units were positive for *Salmonella*, which is a reduction compared to 2007 (0.8%). Germany and Bulgaria reported the majority of the investigations at retail (84.0%); 0.3% and <0.1% of the samples were positive, respectively.

For 2008, no MS reported data from serotyping of ten or more *Salmonella* isolates from eggs and egg products. This corresponds to the generally very low number of *Salmonella* found in eggs in 2008. For previous years some further detailed information is available. In 2007, only five MSs reported *Salmonella* serovar distribution of ten or more isolates. *S*. Enterititis remained the most dominant serovar reported (66.5% of all reported serovars in EU) (EFSA, 2009). For 2006 and according to the CSR (EFSA, 2007a), which presents serotype distribution among isolates in table eggs and egg products for each MS, *S*. Enteritidis is the predominant serovar, with an average of 90.3%.

c. Virulence and antimicrobial resistance patterns

One of the specific criteria to determine *Salmonella* serovars with public health significance is whether any serovars show increased virulence or resistance to relevant therapies for human infections (EC 2160/2003). During the past decades there have been several examples for increased human prevalence and at least partly substantiated increased virulence of certain phage types of *S*. Enteritidis (i.e. PT4, PT8), and *S*. Typhimurium (i.e. DT104, DT193) to animals and/or man. This information has been briefly reviewed and discussed in the previous Scientific Opinion on Quantitative estimation of the impact of setting new target for the reduction of *Salmonella* in breeding hens of *Gallus gallus* (EFSA, 2009), together with the increasing occurrence and changing patterns of antimicrobial resistances among *Salmonella* of human and poultry origin. Most of these changes in virulence and antimicrobial resistance are due to the high genetic flexibility of these bacteria, mainly due to horizontal gene transfer (resulting in acquisition of new genes), triggered by several factors, like changes in population dynamics of the host (animals or man), and changes in therapy practices, and introduction of new antimicrobial drugs. An upcoming EFSA report[11] will provide more information about the patterns and prevalence of antimicrobial resistance among *Salmonella* across the EU.

The recently discovered plasmid-borne, (transmissible) quinolone resistance determinant (*qnr*), representing a new molecular mechanism of quinolone resistance would also need attention as an emerging threat to human health. Several recent reports of *qnr* or its homologues encoded by transferable plasmids in Gram-negative bacteria (including *Salmonella*) highlight the significance of these emerging plasmid-mediated mechanism(s). Besides, *qnr*-plasmids carry integron and multiple resistance determinants including those of beta-lactams and aminoglycosides (Li, 2005, Cattoir and Nordmann, 2009, Garcia-Fernandez 2009). Occurrence of *qnr* genes in *Salmonella* from animals has been so far rarely reported, and no publications have reported *qnr* genes in *Salmonella* from laying hens or eggs.

Another group of recently emerging type is the monophasic group B *Salmonella, S.*4,[5],12:i:- strains, which have been increasing substantially in the EU since 2006 and have also been found in the USA and Canada (Switt et al., 2009). These strains react with the *S.* Typhimurium typing phages and are thought to be derived from *S*. Typhimurium of antigenic structure 4,[5],12:i:1,2. Several phage types have been recognised within such monophasic strains, with DT193 predominating but also phage types U302, U311 and DT120 (Hopkins et al., 2010). Such monophasic *S.* 4,[5],12:i:- strains have been reported from pigs, cattle, poultry and humans. The most common resistance pattern is that of ampicillin, streptomycin, sulphonamides and tetracyclines, which is chromosomally-encoded, but occasionally additional plasmid-mediated resistances have been identified. The organism has been

11 Community Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from animals and food in EU in 2004-2007, to be published in 2010.

found in pigs, cattle and less frequently in poultry in many countries (de la Torre et al., 2003; Sorensen et al., 2002; Zamperini et al., 2007). Other monophasic group B *Salmonella, S.* 4,[5],12:i:- strains with a different resistance gene content have also been identified in several countries. There have been major foodborne outbreaks involving monophasic group B *Salmonella, S.* 4,[5],12:i:- strains in humans in several countries (Agasan et al., 2002; Tavechio et al., 2004; Amavisit et al 2005; Mossong et al., 2007), and monophasic group B *Salmonella, S.* 4,[5],12:i:- were the fifth most common finding in lymph nodes in the EU baseline survey of slaughter pigs that was carried out between 2006 and 2007 (EFSA, 2008).

It has to be pointed out that the BIOHAZ Panel is currently working on an opinion following a request from the European Commission on the monitoring and assessment of the public health risk of the *Salmonella* Typhimurium-like strains (EFSA-Q-2010-00055).

3. Egg production and consumption in the EU

Grand parent and parent flocks are genetically selected on different criteria applicable for the parent and for the progeny such as hatchability, number of eggs produced, colour of shell, conformity of progeny for the type of production (cages, alternatives systems) and the resistance to illness (e.g. against *S.* Enteritidis infection) (Bumstead and Barrow, 1993, Beaumont *et al.* 1999). These birds are vaccinated against poultry diseases, including in some companies against *Salmonella* serovars (*S.* Enteritidis and *S.* Typhimurium), depending on the National Regulations (Barrow and Wallis, 2000; EFSA, 2004). Fertile eggs from these flocks are incubated and hatched in special hatcheries, usually in very good sanitary conditions. After hatching, female day-old chicks are selected, vaccinated and transferred to the rearing farm. During the rearing period, pullets are laid on the floor (litter), for 16 to 18 weeks, then transferred to the laying cycle; this transfer is an important stressful factor for the pullets (vaccination, transport in crates to another building, new environment).

During the laying period (approximately 44 to 52 weeks), the production of eggs increases progressively to reach the optimal production (0.9 egg per day); the quality of the egg (colour, weight, size and conformation) also increases progressively during these first weeks of lay. The management of the flock is mainly to keep hens in good conditions to be able to produce the maximum of eggs. At the end of the laying period, sometimes a moulting procedure of hens can be used for a new period of lay in the same building. More commonly laying hens are transported to the slaughterhouse to be slaughtered in the same manner as broiler flocks.

Figure 1 below presents a chart with the basic egg production chain, from breeding flock to end of cycle.

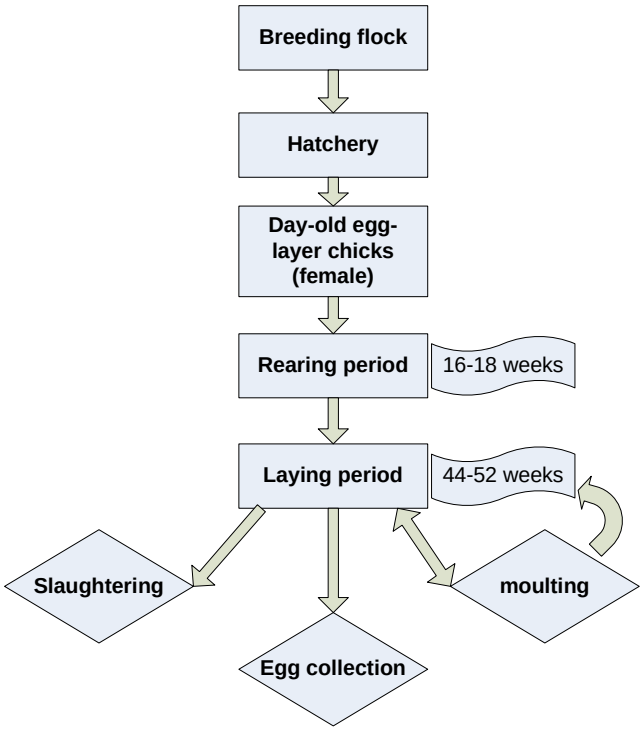


Figure 1: The basic egg production chain, from breeding flock to end of cycle.

Following the new EU Regulation concerning minimum standards for the protection of laying hens (Council Directive 1999/74/EU) banning the conventional cages production, alternative systems have been increasing in some Member States: use of enriched cages following new European standards, barn systems (rearing on the ground, inside the building), free range and organic productions with an access outdoor. The choice of the type of production is clearly different between Member States (see Table 5 for further details).

Table 5: Repartition of laying hens (percentage) in different systems in some EU Member States.

EU MS	Cage (traditional + enriched)	Free Range	Barn	Organic	All alternative systems
France	81	13	3	4	20
Spain	96	2	2	-	4
Germany	63	10	22	5	37
The Netherlands	46	12	40	2	54
U.K.	60	35	5	1	40
1996 (EU-15 MSs)	92	4	4	-	8
2000 (EU-15MSs)	89	6	5	-	11

Source : Institut Technique de l'Aviculture (ITAVI, 2009).

In 2007, in the EU, 85 millions (25 %) of hens were housed in an alternative (non-caged) system. As an example, 17.7 millions of hens (37%) in Germany, and 16.2 millions (54%) in The Netherlands, were housed in alternatives systems, mainly in barn systems. In U.K., the alternative production is mainly based on free range systems. In the opposite end of the spectrum, the Spanish production is mainly based on cage systems (96%). In general, there is a development of barn systems in the EU, representing 56 % of birds reared in alternatives systems. Furthermore, there is also an increase of organic production (7.4 millions of birds in 2007).

During this period, eggs are collected every day, sometimes several times in the day, manually or using an automated belt transporting the eggs until a collecting room. Eggs are selected and stored in a special room before transfer to the packaging centre or to the egg processing (breaking) plant (See Figure 2).

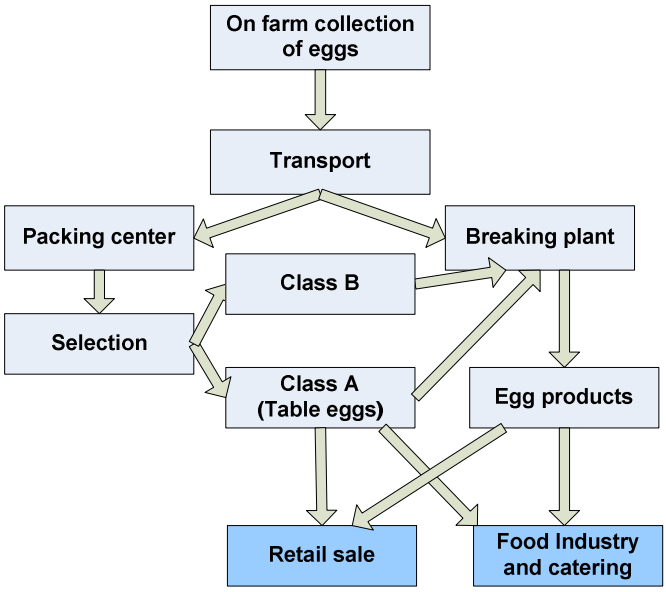


Figure 2: Industrial use of eggs produced in industrial farms.

In 2008, the egg production in the EU was estimated at 6.38 millions tons (104 billions eggs, considering 1kg = 16.4 eggs) (ITAVI, 2009). Almost 75 % of these eggs were produced in 7 MSs (France, Germany, Italy, Spain, United Kingdom, The Netherlands and Poland). This production could be considered as stable (-0.1 % compared to 2007), but this trend seems to be different depending on the MSs (e.g. -5.6 % in Spain and +5.5 % in U.K.). In 2008 and according to data extracted from EUROSTAT[12], the import of eggs and egg products from third countries was reported to be 739,900 tons (67 % to Germany) and the export at 456,700 tons. It has to be noted that third countries exporting eggs to the EU have to comply with all relevant EU legislation, including animal welfare and food safety aspects.

Eggs are very important and complete foods, not only for the nutritional aspect (high-quality proteins, vitamins A, B12, D and E), but also for their functional properties, i.e. the coagulant capacity of proteins, the foaming capacity of albumen proteins, the emulsifying capacity of the yolk, etc. These properties are used in different ways to elaborate and produce many types of varied foods (e.g. bakery products including pastries, meat pies, sauces and dressings, delicatessen, sweets and pasta) and for several (homemade) dishes (e.g. custard and ice cream), the eggs are often used raw or only slightly heat treated. Consequently eggs and egg products are used directly and indirectly in different locations (domestic kitchens, restaurants and caterings, food industries).

Following the EU Regulations (EC) No 589/2008, 'eggs' means eggs in shell — other than broken, incubated or cooked eggs — that are produced by hens of the species *Gallus gallus* and are fit for direct human consumption or for the preparation of egg products. Even if eggs used for these purposes are from laying hens, other birds (e.g. ducks, quails) can also produce eggs for human consumption.

[12] EUROSTAT: http://epp.eurostat.ec.europa.eu/portal/page/portal/eurostat/home/

In the EU, only two classes of eggs, A and B, can be sold since 1st January 2004. The class A category is defined as follow (EU Regulations (EC) No 589/2008 of 23 June 2008):

- shell and cuticle: normal shape, clean and undamaged;
- air space: height not exceeding 6 mm, stationary; however, for eggs to be marketed as 'extra', it may not exceed 4 mm;
- yolk: visible on candling as a shadow only, without clearly discernible outline, slightly mobile upon turning the egg, and returning to a central position;
- white: clear, translucent;
- germ: imperceptible development;
- foreign matter: not permissible;
- foreign smell: not permissible.

Class B eggs shall be eggs which do not meet these characteristics and class A eggs which no longer have those characteristics may be downgraded to class B.

It is therefore no longer be possible to sell class B eggs as table eggs (Council Regulation (EC) No 2052/2003 of 17 November 2003 amending Regulation (EC) No 1907/90 on certain marketing standards for eggs) and these class B eggs can only be sold to industry, not as table eggs, and will be required to be marked as such..

Regarding the Codex Alimentarius document, a table egg is "an egg destined to be sold to the end consumer in its shell and without having received any treatment significantly modifying its properties", and egg product are "all, or a portion of, the contents found inside eggs separated from the shell, with or without added ingredients, intended for human consumption".

Following these regulations one can consider class A eggs as equivalent to "table eggs" and only these eggs are fit for sale to the end consumer.

EU marketing standards and specific public health conditions are set out in Council and Commission legislation. There are specific provisions from farm to consumer, such as eggs being sold to consumers within 21 days of lay. There are also further rules as labeling standards for eggs and packaging (Commission Regulation (EC) No 2295/2003 with later amendments).

Eggs that are not sold as table eggs (class A eggs), either for quality or production issues, can be sent for further processing, which usually aims at prolonging the shelf life of the egg contents without compromising the appreciated functional properties of its components. Due to these functional properties and its ease of use, egg products are also considered to be more convenient, in particular for the food catering industry. Eggs products include processed whole eggs, processed yolks or processed whites separately, with the possible addition of salt and sugar (British Egg Producers Association, 2009). In the EU the egg products production can be estimated at 1.5 million tons, representing less than 25 % of the EU egg production. The main producers are Italy (40 % of the national production), France, The Netherlands and Germany (ITAVI. 2009).

Depending on the type of egg product obtained, whole egg products can be further classified as:

- refrigerated liquid eggs,
- frozen eggs,

- dried eggs and
- cooked eggs.

Further egg products include those obtained from the albumen and yolk separately.

For all these products there are some common processes that are carried out, such as: washing, rinsing, candling of the eggs (i.e. identifying cracked eggs), breaking and separating shells from contents. It is followed by stabilising processes and in most cases (not always) by a pasteurisation treatment. Liquid eggs (or yolks or whites) are produced by pasteurising the egg contents, followed by packaging. The final product has a short shelf life and needs to be kept refrigerated.

The production of frozen eggs is similar to that of liquid eggs, except that the former are not pasteurised but are frozen instead to temperatures below –23.3°C. Once frozen, they can be stored for a long time at suitable temperatures (below –13°C). Dried eggs are produced by spray-drying of pasteurised liquid eggs (whole or yolks). The resulting product has to be stored in cool temperatures (less than 10°C). Cooked egg products are basically precooked eggs and omelettes that are frozen after being produced. Hard boiled eggs are peeled and then packed, usually in a solution with preservatives such as sodium citrate or sodium benzoate. These egg products are mainly used as ingredients in commercial food production, and in the catering industries, restaurants, hospitals and residential facilities.

In 2007, in the EU (25) the consumption of eggs and egg products was estimated at 6 millions tons, meaning an average of 230 eggs eaten per person each year (both table eggs and egg products), with a great variation between MSs (ITAVI, 2009).

4. EU regulated monitoring and control measures of *Salmonella* in laying hens flocks

4.1. Regulatory background

Directive 2003/99 provides for the monitoring of zoonoses in animal populations in the EU. The purpose of this Directive is to ensure that zoonoses, zoonotic agents and related antimicrobial resistance are properly monitored, and that foodborne outbreaks receive proper epidemiological investigation, to enable the collection in the Community of the information necessary to evaluate relevant trends and sources (art. 1). According to article 4, monitoring shall be based on the systems in place in MSs. 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 may be laid down.

The first indications on criteria for *Salmonella* monitoring have been laid down in Regulation (EC) No. 2160/2003, 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 laying flocks of *Gallus gallus* are concerned, the Regulation requires all *Salmonella* strains with public health significance to be monitored, both during the rearing and the laying phase. Samples, to be taken and analysed in the framework of own checks, must include day-old chicks, and flocks two weeks before moving to laying phase or laying unit. In adult laying flocks samples must be taken every 15 weeks during the laying period. No other details are given in Regulation (EC) No. 2160/2003 concerning the kind or number of samples to be taken, or the laboratory methods to be used for the analysis.

Before setting the provisional targets for the reduction of the prevalence of certain *Salmonella* serovars in laying flocks, a baseline study was organised in all EU MSs (Commission Decision 2004/665/EC of 22 September 2004 concerning a baseline study on the prevalence of *Salmonella* in laying flocks of *Gallus gallus*; EC, 2004). One flock per holding was sampled at the end of their

production period by taking five faecal dropping samples and two dust samples. In total, 5,007 laying hen holdings in the EU met the inclusion criteria for the baseline survey.

In general both the observed prevalence for *Salmonella* and *S.* Enteritidis and/or *S.* Typhimurium in MSs in the baseline study was substantially higher compared to the prevalence reported by the MSs for laying hen flocks in the national zoonoses reports for previous years as well as for the regular monitoring results from 2005. This may be caused by a more sensitive sampling design applied in the baseline study. Indeed, the number of samples taken from a flock was generally higher, and the variety of sample material collected greater, than those normally applied in most MSs. The baseline study was performed at the holding level (one flock per holding) resulting in an absolute minimum estimate for the flock prevalence, since negative holdings may, in fact, have had one or more positive flocks that were not sampled.

On the basis of the results of the baseline study, with Regulation (EC) No. 1168/2006, the European Commission has set the targets for the reduction of the prevalence of certain *Salmonella* serovars (*S.* Enteritidis and *S.* Typhimurium) in laying flocks of *Gallus gallus*, and has described the testing scheme necessary to verify their achievement.

Considering the huge differences in prevalence demonstrated in different MSs as a result of the baseline study, two different options were given:

a) an annual minimum percentage of reduction of positive flocks of adult laying hens equal to at least:

   i. 10% if the prevalence in the preceding year was less than 10%;
   ii. 20% if the prevalence in the preceding year was between 10 and 19%;
   iii. 30% if the prevalence in the preceding year was between 20 and 39%;
   iv. 40% if the prevalence in the preceding year was 40%or more;

b) a reduction of the maximum percentage to 2% or less;

However, for MSs with less than 50 flocks of adult laying hens, not more than one adult flock may remain positive.

The sampling frame established in order to monitor the achievement of the targets covers all adult laying flocks which are not meant for domestic use or direct supply. The rearing phase is not comprised in this scheme, and therefore for this phase the only reference is Regulation (EC) No. 2160/2003 (and consequently no official controls are foreseen before the production period).

The food business operator shall take samples at least every fifteen weeks, with the first sampling taking place at the age of 24 ± 2 weeks. Official controls shall consist in sampling at least one flock per year per holding comprising at least 1,000 birds. Furthermore, official controls must be carried out in laying flocks at the age of 24 ± 2 weeks if they were housed in buildings where *Salmonella* was detected in the preceding flock and in any case of suspicion of *S.* Enteritidis or *S.* Typhimurium infection, as a result of the epidemiological investigation of foodborne outbreaks. When *S.* Enteritidis or *S.* Typhimurium are detected in a flock, all other laying flocks on the holding have to be sampled.

The sampling protocol requires that in cage flocks, 2 × 150 grams of naturally pooled faeces shall be taken, whereas in barn or free-range houses the samples consist of two pairs of boot swabs or socks. In the case of sampling by the competent authority, 250 ml containing at least 100 grams of dust shall be collected. If there is not sufficient dust, an additional sample of 150 grams naturally pooled faeces or an additional pair of boot swabs or socks shall be taken.

4.2. Implementation of control programs and results in 2008

In 2008, all MSs except Malta had an approved monitoring and control programme for *Salmonella* in *Gallus gallus* according to Regulation (EC) No 2160/2003 meeting the minimum required sampling requirement set out by Regulation (EC) No 1168/2006. Four MSs have implemented more frequent sampling than every 15th weeks (DK, IE, LT, and SK), one MS (NL) tests blood samples and one MS (DK) is conducting serological testing of eggs from all production flocks. The implemented control measures vary between MSs. In six MSs all serovars are covered by the control programme and in twelve MSs only *S.* Enteritidis and *S.* Typhimurium are included. Restrictions are put on the flock immediately after suspicion in ten MSs and in eight MSs restrictions are placed on egg too.

The consequences for the infected flock vary. Details on the consequences or actions to be applied to both the flock and the eggs vary between the different MSs (See Appendix C for details). Five MSs slaughter infected flocks, five MSs destroy the flocks and two MSs perform sanitary slaughter of the flocks. Five MSs perform sanitary slaughter and destroy the flocks or heat treat products. Four MSs allow treatment with antibiotics, although this may only apply to non-invasive serovars.

The consequence for the eggs can be destruction (4 MSs) or heat treatment (10 MSs). Six MSs apply both strategies. The feed is heat treated or destroyed in five MSs, and the restrictions is placed on the manure in eight MSs. Cleaning and disinfection of the premises is mandatory in 15 MSs and 11 require a negative bacteriological result before restocking. Four MSs require an empty period (drying out) of the premises before replacing the flock. Vaccination is reported to be mandatory in one MS and recommended in two other MSs. In eleven MSs vaccination is permitted and only in three MSs vaccination is prohibited (See Appendix C for details).

Many MSs perform epidemiological investigations of source of infection and tracing of contact flocks in the production system. Intensified examination of other flocks in the holding will also be carried out. Furthermore, in seven MSs the feed suppliers are always included in the investigations.

By 2008, 19 MSs and Norway had already met their reduction targets (see Figure 3 below). Latvia, Luxembourg and Slovenia were above the target in 2008. Target was not set for four MSs (BU, SI, SK, RO). Bulgaria and Romania were not MSs in 2006 and therefore did not participate in the baseline survey that informed the setting of targets for MSs, and Slovakia did not participate in the baseline survey. For these MSs targets will be based on the findings in 2008. Malta did not report results in 2008. Furthermore, 8 MSs plus Norway and Switzerland reported a flock prevalence below 2% for these two serovars, which is the current transitional target.

Overall, approximately 3.5% of laying flocks in the EU was positive for *S.* Enteritidis and/or *S.* Typhimurium at some stage during the production period in 2008 (see Table 6). Bulgaria and Lithuania were the only MSs reporting no positive flocks, and Cyprus only reported other serovars than the two targeted. All other MSs reported between 0.1% and 15.6% samples positive with *S.* Enteritidis and/or *S.* Typhimurium.

In general, more MSs found *Salmonella* in laying hen flocks compared to breeding flocks in the egg production line. This may be because of tighter bio-security at breeding flock level and due to the fact the there has been a mandatory control programme in breeding flocks since 1998 and the targets for breeding flocks had to be met already by 31 December 2009.

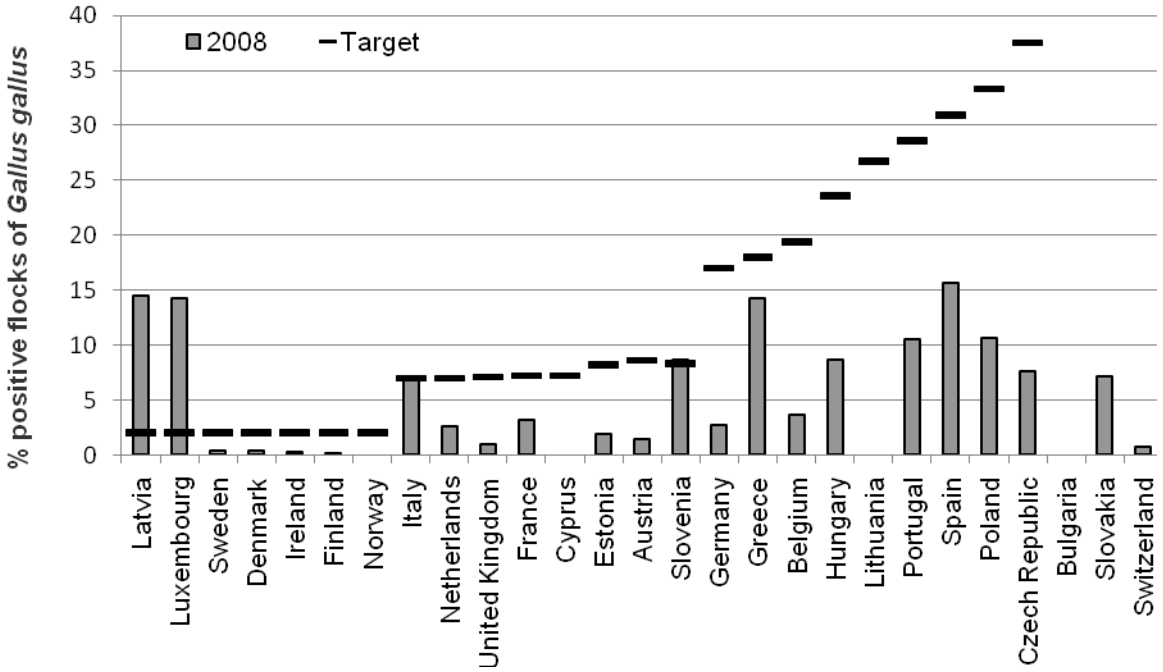


Figure 3: Prevalence of *S.* Enteritidis and *S.* Typhimurium for laying hen flocks of *Gallus gallus* during production period (flock based data) and target for MSs, Switwzerland and Norway, 2008 (EFSA, 2010b). Bulgaria, Rumania and Slovakia did not participate in the baseline survey and thus targets were not set for these MSs. Malta did not report results in 2008.

Table 6: 2008 reported data on *Salmonella* in laying hen flocks of *Gallus gallus* (flock-based data, all age groups) in countries running control programmes in accordance with Regulation (EC) No 1168/2006.

Country	N	Target (production period)	% positive[3]		
			S. Enteritidis	S. Typhimurium	Other serovars, non-typeable, and unspecified
Austria	1,966	8.5	1.2	0.2	1.1
Belgium	649	19.4	3.5	0.2	8.0
Bulgaria[1]	119	-	0	0	0
Cyprus[1]	40	7.2	0	0	12.5
Czech Republic	449	37.5	7.6	0	1.3
Denmark	508	2.0	0.2	0.2	0.2
Estonia[2]	52	8.2	1.9	0	5.8
Finland	950	2.0	0	0.1	0
France	3,067	7.2	2.0	1.2	2.9
Germany[1]	6,304	17.0	2.4	0.4	0.7
Greece[1]	112	23.6	14.3	0	17.0
Hungary	866	23.6	6.7	2.0	3.0
Ireland[1]	326	2.0	0	0.3	0.6
Italy	821	6.8	6.1	0.7	14.0
Latvia	69	2.0	13.0	1.4	5.8
Lithuania[1]	13	26.7	0	0	0
Luxembourg[2]	7	2.0	0	14.3	0
Netherlands[1]	2,346	7.0	2.6	<0.1	0
Poland[2]	1,533	33.3	10.1	0.5	1.9
Portugal[1]	227	28.6	9.7	0.9	21.1
Slovakia[1]	138	-	7.2	0	0
Slovenia	172	8.7	8.7	0	1.7
Spain[1]	845	30.9	13.6	2.0	19.3
Sweden[1]	724	2.0	0	0.4	0.3
United Kingdom	5,523	7.1	0.9	<0.1	0.3
EU total	**27,826**		**3.1**	**0.5**	**2.3**
Norway	1,080	2.0	0	0	0
Switzerland	306	-	0	0.7	0

1. N=Number of units tested since the reported number of existing flocks was not the same as reported number of units.
2. Estonia, Italy, Luxembourg and Poland did not provide information on sampling stage.
3. There is no legal requirement to report occurrence of serovars other than *S.* Enteritidis or *S.* Typhimurium. Thus, values on the last column have to be interpreted with caution.

It is very important for the MSs to implement very strict and rigorous control measures on *Salmonella* suspected and positive flocks. The number of flocks contaminated on an annual basis in each MS is declining, especially in MSs that showed a high prevalence in the baseline study.

The verification of the achievement of the targets is based on the results of the required testing in adult laying flocks, as further detailed in EFSA, 2010b.

5. Epidemiological aspects of *Salmonella* in laying hens

5.1. Patho-biology of different *Salmonella* serovars in laying hens

As discussed in section 2, *S.* Enteritidis is the serovar most frequently associated with egg-related human illness. Among the other serovars, the closest relatives of *S.* Enteritidis are *S.* Gallinarum biovars Pullorum and Gallinarum. The latter is an avian specific non-motile serovar that causes the invasive disease fowl typhoid (Shivaprasad, 2000). Although *S.* Gallinarum is rarely able to cause human disease it is worth remembering that the two pathogenic serovars: *S.* Enteritidis (highly adaptive, motile, non-host specific) and *S.* Gallinarum (avian, non-motile, host restricted) show a high degree of similarity in their LPS-based O antigenic structure (O1, 9, 12) and in their remarkable affinity for avian reproductive tissue. Consequently, they are both capable of infecting avian embryos and eggs. A few decades ago *S.* Gallinarum was a global pathogen causing severe fatal disease of poultry that developed into a life-long carrier status of adults, transmitting infection to the subsequent generations of chicks. Eradication of *S.* Gallinarum is very difficult from an infected farm or hatchery, and for several decades this has been a major goal of the poultry industry. Eradication of *S.* Gallinarum in many countries succeeded by stamping out infected flocks. This was successful because of the limited survival of *S.* Gallinarum in the environment, unlike *S.* Enteritidis, although the more recent tendency of laying farms to use dry cleaning has promoted survival of *S.* Gallinarum in the red-mite population (Parmar and Davies, 2007).

Both *S.* Enteritidis and *S.* Gallinarum belong to the serogroup D1, both are part of the same clonal lineage, and they show a great similarity in their genomic core regions including many of the *Salmonella* pathogenicity islands (SPIs). Both carry fimbrial operons including the one for SEF14 fimbriae which may be important in colonization of reproductive tissue and eggs during egg formation (Turcotte and Woodward, 1993). Although *S.* Enteritidis and *S.* Gallinarum share similarities in pathogenesis and pathobiology of infection, they are different in terms of intestinal colonization and organ invasion: *S.* Enteritidis colonises the chicken intestine and is much less invasive to the organs (liver and spleen) of chicks, consequently causing little or no clinical disease in these animals. Recent comparative genome analysis of *S.* Enteritidis and *S.* Gallinarum has shown that *S.* Gallinarum is a recently evolved non-motile descendant of *S.* Enteritidis, having gone through reductive evolution by deletion and pseudogene formation that has resulted in the strict avian host-specificity of this pathogen (Thomson et al., 2009). In contrast, the genetic content of older and recent isolates of *S.* Enteritidis has been conserved without essential loss of genes. Thus, *S.* Enteritidis also has retained many of the factors that made *S.* Gallinarum a successful vertically-transmitted poultry pathogen. This genetic and phenotypic flexibility might have helped the rapid spread of *S.* Enteritidis in Europe and in the USA via contaminated hatching eggs (Humphrey, 2006). *S.* Enteritidis has also been shown to be more invasive in humans than most other serovars (Hendriksen et al, 2009)

Among other serovars, the second most prevalent human pathogen (at least in the EU) is *S.* Typhimurium representing serogroup B. It however very seldom colonises the ova or oviduct of laying hens, and its route of egg contamination is characteristically egg shell penetration and/or simple surface contamination introduced whilst processing eggs (Cason, 1994). Interestingly the phage type DT104 of *S.* Typhimurium, usually showing a high degree of multidrug resistance and assumed to have an increased pathogenetic potential in humans, does not appear to play a remarkable role in laying flocks and even when present in laying flocks contamination of eggs or egg handling equipment is very rare (Davies, 2003, Carrique-Mas et al., 2009). Certain phage types of *S.* Typhimurium, such as DT2 and DT99 are host-adapted to wild birds (Rabsch et al., 2002) and any infection in laying flocks involving such strains is short-lived and rarely involve ovarian transmission orcontamination of eggs. *S.* Typhimurium of wild-bird origin may be found in free-range flocks, or occasionally in enclosed flocks as a result of feed contamination. These strains represent a smaller public health risk than *S.* Enteritidis. Other sources, e.g. pigs, cattle, companion animals are more prominent in transmission of *S.* Typhimurium. Other serovars i.e. *S.* Mbandaka, *S.* Heidelberg, *S.* Hadar, *S.* Infantis and *S.* Virchow, could also occur with low frequency in layers and consequently on

egg surfaces (Chemaly et al., 2009). Their occurrence varies greatly between continents and countries (Poppe, 1992; Snow et al, 2007; EFSA, 2007). In-ovo infection of table eggs by serovars other than *S.* Gallinarum or *S.* Enteritidis is rare. A possible risk-based approach would not include *S.* Typhimurium and other serovars in the same category as *S.* Enteritidis but could consider alternative approaches such as more frequent faecal sampling. Nevertheless, further detailed data is still needed to fully understand the relevance of these strains.

In the following sections the different stages of the disease in different ages of birds, and the factors influencing the outcome of infection will be focussed on *S.* Enteritidis in layers.

a. Pathogenesis of *Salmonella* infection in poultry (*Gallus gallus*)

The usual three phases of naturally acquired salmonellosis in young chicks are:

- Intestinal colonisation, especially in caecum and on caecal tonsils.
- Invasion beyond the gastrointestinal tract and uptake by macrophages, resulting in multiplication in reticulo-endothelial tissue of the gut-associated lymphoid tissue, liver and spleen (systemic infection) with dissemination into other organs (such as ovaries, especially in case of *S.* Gallinarum and *S.* Enteritidis),
- Clearance, persistence or death: following the establishment of systemic infection the chicken may clear infection through adaptive immunity or infection may persist in small numbers within specific intracellular niches (e.g. in ovaries). In severe cases *Salmonella* continues replication, and extensive bacteriaemia occurs leading to death (Chappell et al., 2009).

The degree and severity of bacterial colonisation, organ invasion, and persistence is highly dependent on serovars, on strains, doses and on the chicken age at the time of infection (Thomas et al., 2009). In case of experimental oral inoculation of freshly hatched chicks, mortality usually reaches a peak level (50-80%) at 3-7 days of age, which can be sharply decreased (3-6%) if infection is delayed until 2 days of age (Gast, 1997). Thus, resistance against clinical disease due to *S.* Enteritidis or other non-typhoidal serovars increases rapidly with the development of the normal intestinal flora and immune system. Mature birds, including layers, do not normally become ill, even with high doses ($log10^7$-10^8) of orally applied *S.* Enteritidis or of other serovars, although bacteriaemia and extensive systemic dissemination have been observed. Predisposing factors (water deprivation, viral-, or coccidial infection, stressful environment, and moulting) may negatively influence resistance of birds to *Salmonella* infection and/or increase the likelihood of earlier infection to reoccur (Gast, 1997). It is also suggested that stress due to different factors could change the biochemistry of the oviduct and favour growth of *Salmonella* leading to a synchronized 'in-ovo' contamination in several hens around the same time (Humphrey et al., 1989).

b. *Salmonella* contamination of the egg contents

The most important public health aspect of *Salmonella* dissemination is contamination of eggs produced, due to colonization of ovaries and oviducts by *S.* Enteritidis. Consequently, the bacteria can intermittently be transferred to the contents of intact, commercially produced, eggs during their formation. This special property of *S.* Enteritidis seems to be the most important factor that has driven the spread and international prevalence of *S.* Eneteritidis especially PT4 (Humphrey et al., 1994). It must be kept in mind that serovars other than *S.* Gallinarum or *S.* Enteritidis (i.e. *S.* Typhimurium, *S.* Heidelberg) have also been shown experimentally to infect eggs by trans-ovarian transmission (Snoeyenbos et al., 1969., Barnhart, et al., 1991.). In fact, a US study could not find a significant difference in *Salmonella* recovery rate from, or localization in reproductive organs of hens orally infected with very high doses of strains of *S.* Enteritidis (PT13a, PT14b), or *S.* Heidelberg (Gast et al., 2007). However, *S.* Enteritidis persists longer and evidence from field investigations suggests that

contamination of egg contents is most frequently the consequence of the infection of reproductive tissue by *S.* Enteritidis.

Using a *S.* Pullorum model it has been shown that in chickens persistently infected with this serovar, bacterial numbers in the spleen increase and infect the reproductive tract as the chicken becomes sexually mature and commences egg laying (Wigley et al., 2001). A similar mechanism is expected for *S.* Enteritidis.

In layers infected with *S.* Enteritidis, colonisation of the reproductive tract is characterised by slight inflammatory processes: focal or diffuse heterophyl infiltration of the reproductive tissues (Hoop and Pospischil, 1993). *Salmonella* is present in egg contents in pure culture (Humphrey, 1994). In flocks naturally infected with *S.* Enteritidis, the incidence of 'in ovo' egg infection is relatively rare (0.06-1.0%) (Humphrey, 2006; Poppe et al., 1992). Egg contamination rates can be higher if birds are experimentally infected with aerosols, especially with *S.* Typhimurium because of higher levels of bacteraemia (Leach et al., 1999). Studies on outbreak-associated eggs have shown that there is no association between the presence of *S.* Enteritidis on egg shells and in egg contents (Humphrey et al., 1998), furthermore the bacterium can be isolated from the reproductive tissues even in the absence of intestinal carriage (Bygrave and Gallagher, 1989). It seems that there could be differences between European (mostly PT4) and US (mostly non-PT4) isolates of *S.* Enteritidis regarding the principal site of reproductive tract for infection.

Egg contamination may also be induced intravaginally, as shown by experimental ascending infection studies due to *S.* Enteritidis or other serovars (i.e. *S.* Typhimurium, *S.* Infantis, *S.* Hadar, *S.* Heidelberg or *S.* Montevideo). The ability to colonise the reproductive organs of chickens and to contaminate eggs was significantly higher for *S.* Enteritidis than the other serovars, and especially the inner shell was contaminated with *S.* Enteritidis organisms (in 10 of 40 eggs). The contamination rates and the viable counts in the cloaca were also significantly higher in hens inoculated with *S.* Enteritidis than in those inoculated with the other serovars at 4 days post-inoculation (PI), while the caeca were colonized similarly by each serovar at 7 days PI. Comparison of in vitro adherence of these six serovars using vaginal explants has also confirmed that *S.* Enteritidis has a specific advantage over the other *Salmonella* serovars in its capacity to colonise the vaginal tissues of hens, and this higher affinity to the vagina is likely to play a major role in the production of *S.* Enteritidis contaminated eggs (Okamura et al., 2001). These advantages of *S.* Enteritidis in long term colonisation of the reproductive organs of hens have been confirmed using different approaches and somewhat different strains (Okamura et al., 2001, Mizumoto, et al., 2005). Furthermore, it has been established that the mean number of *Salmonella* in the vaginal epithelium depended on their lipopolysaccharide (LPS) type, with the rank order as follows: LPS type O9 (*S.* Enteritidis) > LPS type O4 (*S.* Agona, *S.* Typhimurium and *S.* Heidelberg) > LPS type O7 (*S.* Montevideo and *S.* Infantis) and LPS type O8 (*S.* Hadar). This rank order of *Salmonella* invasiveness is in accordance with the frequency of *Salmonella* outbreaks involving contaminated eggs (Mizumoto et al., 2005).

The reasons for this kind of reproductive-tissue tropism of *S.* Enteritidis are not well understood. It is assumed that the SEF14 fimbriae, produced by both *S.* Entertidis and *S.* Gallinarum, may be involved in reproductive tissue colonisation (Turcotte and Woodward, 1993), and the enhanced survival at 42ºC may also allow *S.* Enteritidis to contaminate egg contents more successfully than other serovars such as *S.* Typhimurium, *S.* Hadar, *S.* Heidelberg, and *S.* Virchow (Gantois et al., 2008). The study on roles of the two important type-three secretion systems (T3SS-1 and T3SS-2, encoded on SPI-1 and SPI-2) during *S.* Enteritidis infection of primary chicken oviduct epithelial cells (COEC) and macrophages has shown that both T3SS are required by *S.* Enteritidis to invade oviduct cells and to survive in these and in peripheral blood derived macrophages (Li et al., 2009). *S.* Enteritidis also has the ability to alter the cell surface dramatically; it frequently produces glycosylated high-molecular-mass LPS that may be important for persistence in eggs and may assist the efficiency of transmission of infection to man (Jean Guard-Petter, 2001). *S.* Enteritidis fimbrial protein (SefA) is also involved in the superior ability of *S.* Enteritidis to attach to ovarian granulosa cells and other cells (Rank et al.,

2009). All these findings suggest that *S.* Enteritidis has a higher ability to colonise the reproductive epithelium than other serovars, and that the *Salmonella* LPS type may play an essential role in tropism of the reproductive tract. There is also a very complex interaction between *S.* Enteritidis and the oviductal immune response that may help facilitate long-term colonisation (Li et al., 2009). These and several other data provide evidence that altered bacterial growth patterns and specific cell surface characteristics contribute to the adaptation of *S.* Enteritidis to the avian reproductive environment. Beside other factors discussed in chapters below, this could also explain why *S.* Enteritidis is most frequently involved in contamination of egg content of layers, and thus why it represents a major threat for public health (Humphrey et al., 1991).

In conclusion, egg contamination with *S.* Enteritidis occurs whilst the forming egg is in the reproductive tract, but only rarely the bacterium can be recovered from egg contents laid by infected hens. On flock level, in general, internal-egg contamination levels of 1% or less have been described. However, this has varied greatly among surveys and trials with experimentally infected flocks, due to several factors.

c. Resistance and immune response of poultry to *Salmonella* Enteritidis with special regard to laying hens

The basis for resistance to *Salmonella* infection lays in the two broad categories of immunity: non-specific (innate) and specific (acquired) immunity. The outcome of infection is a result of combined action of both categories and the results may also vary between particular genetic lines of chicks. For instance, genetic resistance to systemic salmonellosis in the chicken is encoded by a number of factors including the major histocompatibility complex Nramp1 (now termed Slc11a1) and a gene, SAL1 that leads to increased macrophage activity against *Salmonella*. These genes are located on two different chromosomes of *Gallus gallus*. Studies in outbred and, in particular, inbred chickens have revealed considerable differences in levels of colonization of the gastrointestinal tract and responses to vaccination (Reviewed by Wigley, 2004). It appears, however, that selective breeding for increased genetic resistance to *Salmonella* would be difficult to harmonize with the genetics of increased production traits and with simultaneous resistance to several other poultry pathogens. Therefore, increasing the acquired immunity through vaccines seems to be a more realistic approach in controlling *Salmonella* in poultry, including layers (Reviewed by Van Immerseel et al., 2005).

Wigley et al. (2005) have demonstrated that at the onset of laying both the T-cell response to *Salmonella* and non-specific responses to mitogenic stimulation fall sharply in both infected and non-infected birds. The fall in T-cell responsiveness coincides with the increase in numbers of *S.* Pullorum and its spread to the reproductive tract. Three weeks after the onset of egg laying, T-cell responsiveness began to increase again and bacterial numbers declined. Specific antibody levels changed little at the onset of laying but increased following the rise in bacterial numbers in a manner reminiscent of a secondary antibody response to rechallenge. These findings indicate that a non-specific suppression of cellular responses occurs at the onset of laying and plays a major role the ability of *Salmonella* (in this case: serovar Pullorum) to infect the reproductive tract, leading to transmission to eggs. Stress hormones may help increasing bacterial growth in the oviduct (Burton et al., 2002).

d. Vaccination of domestic fowl against *Salmonella*

The first experiences about successful vaccines against *Salmonella* in poultry were gained in the immunization against fowl typhoid (*S.* Gallinarum/Pullorum infection). Such vaccines are not required or recommended in industrialized countries where the infection has been eradicated or occurs only infrequently, but it is recommended in countries where the disease is still prevalent. During the last three decades, several experimental and commercial (live oral or injectable killed) vaccines have been described as successfully reducing spread of infection and clinical disease (Barrow et al., 1990.,

Griffin et al., 1993). Among them the most established vaccine is the 9R strain of *S*. Gallinarum that is also commercially available (Gupta et al., 1977).

The first vaccines against non-typhoid poultry salmonellosis were partly used based on these experiences, partly based on the successful use of *Salmonella* vaccines in animals other than poultry in the 1980ies in East-Germany with the main goal to reduce *Salmonella* contamination of poultry products and thereby to protect human health (Meyer et al. 1993). As described in a previous EFSA Scientific Opinion related to the use of vaccines for the control of *Salmonella* in poultry (EFSA, 2004), in several countries vaccination against *S*. Enteritidis and *S*. Typhimurium has now been used widely in parent breeders and commercial laying hens for many years (see Table 3 in Appendix C). Vaccination is only regarded as an additional measure to increase resistance of chicks against *Salmonella*, especially if the flock prevalence is high. Although such vaccination is not fully protective, especially in the case of laying hens placed in a previously contaminated laying house, it is likely to reduce fecal shedding, ovarial transmission, and the within flock-prevalence, thereby reducing contamination of table eggs and the environment. Most importantly, the use of vaccination against *S*. Enteritidis and *S*. Typhimurium seems to lower internal-egg contamination levels (Davies and Breslin, 2004), thereby most directly contributing to public health. Inactivated injectable vaccines may lead to maternal antibodies being transmitted in eggs that may also reduce the establishment of early infection in chicks placed in a contaminated environment (Inoue et al, 2008). It is widely accepted that *Salmonella* live vaccines confer better protection than killed vaccines, because the former stimulate both cell-mediated and humoral immunity. However, due to food safety requirements, such live vaccines are not permitted for use in layers. Apart from that, MSs apply vaccination to different extents (or not at all), according to their specific national control programs to prevent *Salmonella* contamination of foodstuffs (EFSA, 2004).

5.2. Dynamics of *Salmonella* infection in the laying hen flock

Infection dynamics of *S.* Enteritidis may depend on a number of factors (e.g. housing system, flock management). Several epidemiological studies have been published, amongst them observational studies aiming to identify risk factors for introduction of *Salmonella* in laying hen flocks. Risk factors (RFs) concerning flock characteristics were flock size (Mollenhorst et al., 2005; Namata et al., 2008; EFSA, 2007; Huneau-Salaün et al., 2009), and flock age (Garber et al., 2003; EFSA, 2007; Namata et al., 2008). Farm management and type of housing were also identified as RFs. Both on-floor systems (Garber et al., 2003; Mollenhorst et al, 2005) and cage systems (EFSA, 2007; Namata et al., 2008) were found to increase the risk of colonization. This is also true in multi-stage management in on-floor flocks (Mollenhorst et al., 2005; Huneau- Salaün et al., 2009). Other factors were the occurrence of colonization of the previous flock in on-floor flocks (Huneau-Salaün et al., 2009), the absence of cleaning and disinfection of the poultry house between subsequent production cycles (Garber et al., 2003) and entry of delivery trucks near poultry house entrance (Huneau-Salaün et al., 2009). In addition, it appeared that seasonality was associated with serotypes other than *S.* Enteritidis and *S.* Typhimurium (EFSA, 2006). Vaccination of hens against *Salmonella* was a protective factor, except for *S.* Typhimurium (EFSA, 2007). It should be noted that the studies by Garber et al. (2003) and Mollenhorst et al. (2005) took only *S.* Enteritidis into account.

Four MSs used data from the 2004/2005 EU baseline survey of *Salmonella* in laying hens for identification of RFs. In Belgium, RFs for presence of *Salmonella* in laying hen flocks were; cage systems, flock size and flock age (Namata et al., 2008). In a follow-up study in the UK, it was the level of *Salmonella* contamination of the previous flock (Carrique-Mas et al., 2008a). In Austria, the factor associated with *Salmonella* status was cage housing (Much et al., 2007). In France, RFs associated with *Salmonella* contamination of eggshells were; entry of delivery trucks near the poultry house entrance, flock size, more than five culture positive environmental samples, high egg-laying rate and mixed farming (Chemaly et al., 2009).

In a cross sectional study performed in four EU MSs (Belgium, Germany, Greece and Italy) plus Switzerland, RFs associated with shedding of *S.* Enteritids or *S.* Typhimurium were: housing in conventional battery cages, the absence of dry cleaning in between production rounds, and sampling in winter (van Hoorebeke et al., 2010).

New EU welfare legislation that will lead to a ban on the use of conventional 'battery' cages for laying hens in 2012 will mean that cage houses will have to be either decommissioned or refurbished to provide an alternative housing system. Such alternative systems that involve smaller flocks are less conducive to *Salmonella* infection. Some cage houses may be converted to barn production, typically as two storey barns but the most likely option is conversion to enriched colony cages, in which groups of up to 60 birds are housed in a larger cage that provides more space, perches and a 'nest-box' area. Conversion of houses will require removal of old-style cages and this offers an excellent opportunity to eliminate farm pests that can carry *Salmonella*, such as rodents and litter beetles, as well as red-mites which can reduce the resistance of birds to *Salmonella* infection in the case of heavy infestations. During the extended down-time involved in refurbishment houses can be deep cleaned and intensively disinfected to remove residual environmental contamination. This is a great opportunity to eliminate resident *Salmonella* from cage houses that should not be missed. Colony cages are typically easier to clean that conventional cages and the belt-clean system means that there is less harbourage for rodents and flies than in deep pit houses that they replace. This should also help to minimise the risk of recontamination of these houses but it is important that producers apply a high standard of within-holding bio-security to prevent spread of infection from older flocks to new flocks placed in refurbished houses.

Despite the fact that numerous RFs associated with colonization have been identified and quantified, and several control measures have been implemented, introduction of *Salmonella* into flocks still occurs, though at a lower frequency than before (e.g. Van de Giessen et al., 2006). The rate of transmission of *Salmonella* within a flock determines the change in within-flock prevalence, which, in turn determines when a colonised flock can be detected.

After introduction into a flock, transmission of *Salmonella* between hens occurs via contact with infected individuals and ingestion of faecally contaminated materials (Holt et al., 1998), feed and water (Nakamura et al., 1997, 1994; Holt, 1995), and aerosols (Baskerville et al., 1992; Nakamura et al., 1997; Gast et al., 1998; Holt et al., 1998). The potential for contact transmission of *Salmonella* may be improved when birds are subjected to stress, especially induced moulting (e.g. Holt and Porter, 1992; Holt, 1995; Holt et al., 1998). All studies mentioned above were carried out with hens housed in wire-floored cages and used high experimental doses.

After colonisation, individual laying hens shed *Salmonella* in their faeces intermittently, as measured by classical culture methods. Most hens stop shedding the bacteria after approximately three weeks (Shivaprasad, 1990; Gast et al., 2005). However, under stress (water deprivation, viral or coccidial infection, stressful environment, and moulting) the hens might start shedding again (Skov et al., 2002). This could be explained by reactivation of shedding (Barrow, 1992) or to a higher susceptibility to re-infection from the environment (Skov, 2002) as especially *S.* Enteritidis has a tendency to have long-term persistence in laying houses possibly related to rodent levels and housing system (Carrique-Mas et al., 2008b). It follows that once *S.* Enteritidis is introduced into a flock, it can maintain throughout a whole production period (but over time the number of organisms excreted by infected birds, and as a consequence, the within-flock prevalence, may decrease). Detailed data on how the prevalence of *S.* Enteritidis is likely to change over the lifetime of a flock is not available (Arnold et al., 2009a).

There are several limitations to the transmission studies mentioned earlier. One is that only cage systems are used, whereas on-floor or barn production systems are increasingly important in Europe. Another limitation is that they present only qualitative information about transmission of *S.* Enteritidis with key questions unsolved: does *S.* Enteritidis spread in the flock or not, and if so, via which route?

It is however important to have knowledge about the rate of transmission in the flock as well. First of all, this knowledge is helpful to determine via back calculation when bacteria were introduced into the flock from change in prevalence over time. This could help to identify high risk periods more precisely (Bos et al., 2007; Van Gerwe et al., 2009) than currently known, namely, flock age being a risk factor (Garber et al., 2003; EFSA, 2007; Namata et al., 2008). The rate of transmission also determines when after colonisation of the first layer a flock can be detected as colonised using a fixed sample size (Van Gerwe et al., 2009).

Important parameters used for quantification of transmission are the basic reproduction ratio R_0, defined as the average number of secondary cases caused by one typical infectious case in a fully susceptible population, and the transmission rate parameter β, defined as the number of new infections that occur due to one infectious animal per unit of time.

Quantitative epidemiological information about horizontal transmission of *S.* Enteritidis is presented by Thomas et al. (2009). These authors quantified transmission of *S.* Enteritidis in pairs of laying hens, housed in litter cages. The mean initial transmission rate β was estimated to be 0.47 day^{-1} [0.30; 0.72] and the R_0 to be 2.8 [1.9; 4.2]. Experiments under field conditions on *Salmonella* dynamics in commercial flocks are necessary to validate these findings, as they could be of importance for modelling, surveillance and control.

6. Detection of *Salmonella* in the laying hen flock

6.1. Sampling methods

The efficiency of sampling programmes has a large effect on the detection of *Salmonella* and therefore estimation of prevalence (Fletcher 2006) and it is difficult to design an optimal sample size when the within-flock prevalence and number of organisms is unknown (Altekruse et al 2003). Such considerations are key to defining an effective sampling programme to verify the anticipated reduction of *Salmonella* in flocks of laying hens (Frank et al 2009) in response to EU regulations and targets.

There are numerous sampling methods available. In many countries litter has traditionally been used for non-cage flocks. Litter can be a good sample if it is taken in a diligent and representative way by sub-sampling throughout the house, but this is seldom done correctly by Food Business Operators (FBOs) and so other samples such as bootswabs may perform better (Kingston 1981). Another popular sampling method is cloacal swabs but this method is relatively insensitive unless very large numbers of samples are taken. Sampling caecal and/or ovary/oviduct samples from birds at post-mortem is considered to be a definitive test (Nief and Hoop 1998) but a large number of birds (e.g. 300 to detect 1%) is needed to detect a low within-flock prevalence (Barnhart et al 1993).

In the egg industry many consider that the prevalence of *Salmonella* in egg, and specifically egg contents, is the most relevant measure of public health risk but the prevalence of contaminated eggs, even from a flock or bird known to be infected with *S.* Enteritidis, can be very low (Gast, 1993; Gast and Holt, 1998), making meaningful egg testing very laborious and expensive.

It has been recognised for some time that thorough environmental sampling is usually the most effective way to detect *Salmonella* in a poultry flock (Aho, 1992; Johansson et al., 1996; Musgrove and Jones, 2005) and normally the occurrence of *Salmonella* in the occupied part a house reflects infection in the flock (Arnold et al., 2009a).

An intermediate stage between collection of cloacal swabs directly from the birds and environmental sampling is collection of fresh faeces. This can be done by collecting naturally pooled material or by collecting and pooling individual voided faeces. The inclusion of larger volumes of mixed faecal material from a large number of birds enhances detection (Wales et al., 2006) by increasing the

chance of including faeces from a high-level shedder (Hildebrandt and Bohmer, 1998; Arnold et al., 2005; Arnold et al., 2009b). There is however a risk that minority strains of *Salmonella* may fail to be detected in a mixed population of strains, including live vaccine, in the sample (Kinde et al., 1996).

Manual collection of individual droppings to create pooled faecal samples is laborious and two main methods have been described to overcome this. Drag swabs were developed in the USA (White et al., 1997; Castellan et al., 2004) to sample very large poultry houses. Each drag swab usually comprises at least three separate 100 cm2 moist surgical gauze swabs (Mallinson et al., 1989) that are dragged behind an operator who walks the length of the houses. This method proved more effective than limited litter sampling (Kingston, 1981), The performance of drag-swabs can be substantially improved by intermittently stepping on the swabs during sampling (Buhr et al., 2007).

There has been a substantial amount of literature comparing boot or sock swabs with drag swabs. Sock swabs, comprising a folded section of tubegrip bandage applied over the ball of the foot of the sampler and turned four times during sampling, were first described in Denmark (Skov et al., 1999). In the USA boot swabs utilising gauze surgical shoe covers were used and found to be superior to drag swabs (Caldwell et al., 1998; McCrea et al., 2005; McCrea et al., 2008) even when the method of stepping on drag swabs was used (Buhr et al., 2007).

Large hand-held gauze (or 'chiffonette') swabs can also be effective for sampling (Davies and Wray, 1996a; Carrique-Mas and Davies, 2008; Zewde et al., 2009) but require more effort and dedication to achieve a representative sample.

Dust is a useful sample for identifying recent excretion of *Salmonella* in a poultry flock (Riemann et al., 1998). It is normally best to take both fresh faecal and dust samples (Davies and Wray., 1996a) to help compensate for variable detection in either sample.

Immunological detection by serology can also be used to identify indirect evidence of likely exposure to *Salmonella* by detecting antibodies in serum or egg yolk (Davies et al., 1997; Feld et al., 2000). This increases the sensitivity of detection of those serotypes included in the ELISA based test, normally *S.* Enteritidis and *S.* Typhimurium, compared with bacteriology alone and a combination testing programme has been successfully used in Denmark for many years (Wegener et al 2003). Such testing can not readily be used in vaccinated flocks but is a useful additional voluntary measure in non-vaccinated flocks. Serological testing can also give useful guidance even in flocks that have received live vaccines by the oral route, as this typically only elicits a transient low-level response so birds with high titres are likely to have been exposed to field infection. Serological testing also frequently detects false positive reactions caused by exposure of birds to organisms with antigens that are shared with the target organisms so can only be used as an adjunct to bacteriological monitoring in cases where radical control action must be taken, and high serological cut-off levels have to be designated, which limits detection of low-titre positive birds. Ideally serological tests should be able to differentiate vaccinal from field reactions but this is not possible with current *S.* Enteritidis / *S.* Typhimurium vaccines as these have no serological markers in terms of missing or additional antigens. It is unlikely that there will be extensive regulatory use of serological monitoring in the future when EU control programmes are based on bacteriological testing.

6.2. Sampling and test methods used in the baseline survey and for routine monitoring in the EU

Sampling of laying flocks for the baseline survey of flocks of laying hens was problematic because of the different housing systems involved. The objective was to detect with 95% confidence flocks with a 1% within-flock prevalence (EC 2004) as such low levels of infection were expected, especially in vaccinated flocks of mature, healthy birds. The calculated number of samples for this was 300 individual faeces. Since it was impractical to collect 300 faeces from each flock in the survey this was extrapolated to taking at least 300g of naturally mixed faecal material. From cage flocks 5 x 200g

samples were taken, thoroughly mixed and sub-sampled to provide a considerable margin for error. In non-cage flocks five pairs of boot or sock swabs were taken, based on the finding that in Danish broiler flocks this was equivalent to 300 individual faeces, each cultured as part of a 5g pool involving 60 separate cultures in total (Skov et al., 1999). This took no account of the different housing systems used for laying flocks compared with broilers, the age of the birds, number of organisms likely to be excreted (Jordan et al., 2004), or use of vaccination. On the other hand the ISO 6579 Annex D culture method used for the survey is likely to have been more sensitive than the NMKL-71 method used in the Danish study (Voogt et al., 2001; Eriksson and Aspan., 2007). The addition of two large samples of dust to the baseline survey method was considered to provide maximum detection potential given the constraints of a maximum limit of seven samples.

Following the EU baseline survey carried out in 2007, a decision was made to use routine monitoring results as the basis for assessing achievement of the *Salmonella* reduction target rather than repeating the baseline and it was agreed that a single large faeces sample test or two pairs of boot swabs cultured as one sample could be used for operator monitoring. Two pairs of boot swabs is considered to be equivalent to at least 60 faecal droppings samples cultured as one pool (Gradel et al., 2002; Ellerbroek et al., 2002) so reliably detecting a 5% prevalence on a single sampling occasion. Comparative studies of the various sampling options suggested that operator sampling carried out correctly could be more sensitive than this and cumulatively three sampling rounds could given equivalent sensitivity to the full baseline survey protocol (Carrique-Mas et al., 2008a). The addition of dust to the official sampling, and official sampling of all other flocks on a positive holding as well as new flocks placed in positive houses should further enhance the sensitivity of detection but there could still be influence of systematic false negative results due to factors such as difficult-to sample-houses, poor sampling technique by operators, clearance of dust before official testing, delays between sampling and testing and effect of the efficiency of testing laboratories. A combination of such factors means that the identification of positive flocks by the monitoring programme is likely to be significantly less than in the baseline survey. It may be possible to attempt to assess the magnitude of under-detection by comparing the results of operator and official sampling. A recent paper by Arnold and colleagues suggests that it would be useful to compare results from official and operator's samples to verify performance of the sampling plans (Arnold et al., 2010).

The sensitivity of the three optional confirmatory test methods is related to either detection of a 1% within-flock prevalence (300 bird test), equivalent test to that used in the baseline survey or to negative results obtained from testing 4,000 eggs as 100 pools of 40 eggs. The egg test is based on detection of prevalence of 0.06% positive eggs (including shells), a figure relating to findings of a retail egg survey carried out in the UK in 2003 (FSA 2004, Commission Regulation (EC) No. 1237/2007).

6.3. Factors influencing detection of *Salmonella* infected flocks

a. Flock housing/manure handling system

The reports of the baseline survey, and other related papers (Methner et al., 2006; Snow et al., 2007; Much et al., 2007) have shown an increased risk of detection of *Salmonella*, mainly *S.* Enteritidis, in cage flocks. It is not clear whether some of this reported difference may relate to the different sampling methods and the different sources of replacement birds. Within cage systems deep pit manure removal has been associated with increased detection and persistence of infection (Carrique-Mas et al., 2009). Under-detection of *Salmonella,* compared with intensive sampling using 20-40 Chiffonette samples per house, was greatest in non-cage houses and step cage houses because of the difficulty in obtaining representative samples (Carrique-Mas et al., 2008a). Dust samples are more variable in non-cage systems and do not always increase detection compared with additional boot swabs (Mahe et al., 2008). The role of rodents is critical in maintenance of infection on farms and

vaccinated flocks may clear infection, or achieve a very low within-flock prevalence within weeks when breeding rodent populations are eliminated (Carrique-Mas et al., 2009).

b. Flock size

The baseline survey and various related studies cited above also detected an increased risk of identifying positive flocks as flock size increases (Mollenhorst et al., 2005). Flock size is often closely related to the type of housing system and only cage houses are likely to hold more than 30,000 birds. Large flocks are also more likely to be held on large holdings with multi-age production, which is another risk factor. Studies in UK have suggested that within-flock prevalence may be lower in very large holdings (Carrique-Mas et al., 2008b) especially in large cage houses or large subdivided non-cage housing, making detection more difficult. It can however be difficult to fully distinguish between the influence of difficulties in representatively sampling a particular type of laying house from factors that influence the within-flock prevalence of birds in the house. This in turn affects the likelihood of detection (Arnold et al., 2009a) so can have an influence on the flock prevalence reported in surveys.

c. Stage of lay

Baseline surveys and other studies have shown an increased tendency for flocks to be identified as *Salmonella*-positive as the birds become older, i.e. during late lay (Garber et al., 2003; van de Giessen et al., 2006; Wales et al., 2007), especially if birds have been moulted (Golden et al., 2008). In most cases the initial infection derived from residual contamination of laying houses occurs on placing pullets that are suffering from transport, handling and relocation/remixing stress at a time when hormonal changes associated with the onset of lay are also increasing susceptibility to infection (Line et al., 1997). This leads to a typical early peak of infection within three weeks of housing (Humbert et al 1995; Gradel et al 2002) but laying flocks are rarely sampled at this time (16-19 weeks of age). After this early peak excretion of *Salmonella* typically subsides, making detection more difficult especially if the 1st sampling is delayed until 24±2 weeks as currently prescribed in EU regulations. Subsequently there may be an increase of excretion towards the end of lay, but in the absence of rodents this is less likely to occur and infection may spontaneously resolve (Carrique-Mas et al., 2009). This is an example of genuine under-detection of positive flocks which are sampled between the early and late peaks of excretion of *S.* Enteritidis.

Improved detection of *S.* Enteritidis positive flocks could be achieved by effective sampling and early detection. This could be done if the first operator sampling as well as official control sampling were to be carried out 2-3 weeks after entry of birds to the laying house (e.g. at 20 weeks of age), as infection with *S.* Enteritidis is most readily detected in the 2-3 week period following transfer of birds to the laying house. However, there is the possibility that early infections with serovars other than *S.* Enteritidis, including *S.* Typhimurium, could peak early in the life of a flock but then could regress and resolve (Humbert et al., 1995, Gradel et al., 2002). Recent work in UK and USA has also shown that many cases of early *S.* Enteritidis infection in vaccinated flocks where there is good rodent control and hygiene in the laying house (e.g. regular clearance of dust and accumulated faeces) may resolve spontaneously by 22 - 26 weeks (Carrique-Mas et al., 2009). This in contrast to older data from experimentally infected, day old, unvaccinated chicks in which immune tolerance may lead to development a life-long carrier state in a proportion of birds (Holt et al. 1999).

d. Vaccination

Both live and inactivated vaccines are available for control of *S.* Enteritidis and/or *S.* Typhimurium. The protection offered by vaccination is often not complete or sustained and although the likelihood of infection of eggs is reduced (Davies and Breslin, 2003b) detection of infected flocks may also be reduced as a result of reduction of the within-flock prevalence and number of organisms shed in faeces (Van Immerseel et al., 2004; Gantois et al., 2006; Inoue et al., 2008). Waning of vaccinal protection may be involved in the rise in excretion towards the end of lay.

6.4. Significance of under-detection of *Salmonella* positive flocks

The various factors mentioned above, as well as use of competitive exclusion products, organic acids in feed, or especially water, and therapeutic antimicrobials, can lead to failure to detect an infected flock (Seo et al., 2000; Davies and Breslin., 2003b; Jarquin et al., 2007; Vila et al., 2009). Intermittent excretion and low within-flock prevalences (Desmidt et al., 1997) present challenges and more intensive sampling has demonstrated potential limitations of procedures used for baseline surveys and routine monitoring of laying flocks in the control programme (Carrique-Mas et al., 2008a; Carrique-Mas et al., 2008b; Arnold et al., 2009b; Van Hoorebeke et al., 2009). Non-uniform distribution of infection and contamination of laying houses, and the difficulty of representative sampling in large complex housing systems, makes detection more problematic (Riemann et al., 1998; Hayes et al., 2000; Rolfe et al., 2000). Further consideration has to be given to the possible use of antimicrobials, which may affect detection of truly positive flocks.

During testing of samples competing organisms are the main limiting factor in detection (Arroyo and Arroyo, 1995) and it is important to allow enough free enrichment media around the sample for *Salmonella* to migrate away from the sample matrix (McCrea et al., 2005). Low numbers of *Salmonella* organisms, e.g. less than 10 cfu/g, can be especially difficult to identify against an overwhelming background of other organisms (Cox and Berrang, 2000), including some live vaccine strains.

There are also potential issues with auto-agglutinating (rough) ('O' rough:gm:-) or monophasic strains of *S.* Enteritidis (9,12:-:-) and rough strains of *S.* Typhimurium ('O' rough:i:1,2). When these strains occur is an indication that *S.* Enteritidis or *S.* Typhimurium is likely to be present (Sonne-Hansen and Jenabian) and the flock should be treated as suspect and re-sampled.

The application of more intensive sampling than the standard official sampling on laying hen farms where a link to human salmonellosis cases is to be investigated, in order to increase the chances of detecting flock infection, should be recommended. As an example, the methodology employed in the EU baseline survey, or an even more sensitive method (Carrique-Mas et al 2008a), could be used for all flocks on the holding. Farm-based egg packing facilities could be included in the sampling. This is very important as a single very highly infected flock can lead to a large number of human cases. A good example of this is described in a recently published report of the UK Health Protection Agency where eggs contaminated with a quinolone resistant strain of *S.* Enteritidis PT 14b from a single flock that were imported into UK during 2009 and used in catering establishments caused at least 152 reported cases amongst a wider outbreak of 489 possible cases (HPA, 2010). If one flock is infected on a holding, it is likely that other flocks will also be infected (Carrique-Mas et al., 2008b), so a thorough epidemiological investigation should be implemented.

Overall, it is clear that detection of *Salmonella* in laying hen flocks is far from straightforward and some flocks will fail to be detected by any method, including the baseline survey and control programme methods. The significance of this failure to detect (i.e. false negatives) in terms of public health is unclear, since it is the most highly infected flocks that are likely to be detected, and eggs from flocks with low levels of infection are less likely to be contaminated (Van Hoorebeke et al., 2009).

7. *Salmonella* contamination of the table egg for human consumption

The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union (CSR) in 2008 gathers data on *Salmonella* prevalence in table eggs for several EU Member States (MSs) (EFSA, 2009). The prevalence based on single or batch sampling testing for different countries has been estimated to range from 0% (in 10 MSs) to 22.6%. The average *Salmonella* prevalence was 0.4%., which is half the level found in 2007. The sampling procedures and microbiological analysis are not standardised between MSs.

These data present in the CSR are in line with other studies carried out in the UK (ACMSF, 1993: Report on *Salmonella* in eggs: 0.6% of table eggs; ACMSF, 2001: Second report on *Salmonella* in eggs: 0.99% of table eggs). Most studies have found a higher prevalence of shell contamination compared to that of the egg contents (FSA, 2004; Davies and Breslin, 2004; FSA, 2007; Murchie et al., 2007). Studies conducted with large number of eggs show similar results (Table 7). There appears to be no relationship between either contamination of the eggshell or faecal carriage and the presence of *Salmonella* in the egg content (Humphrey, 1994).

Table 7: Findings on *Salmonella* serovars in studies sampling large number of eggs.

No of *Salmonella* Enteritidis positive samples		**No of non *Salmonella* Enteritidis positive samples**		**No of samples**	**Country**	**Source**
Contents	**Shells**	**Contents**	**Shells**			
1	2	0	6	12,540	Northern Ireland	Wilson et al., 1998
16	103	2	17	83,820	UK	Wall and Ward, 1999
8	149	29	4	1,744	Various EU MSs	FSA, 2006
1	5	0	1	9,402	UK	FSA, 2007
0	n/a	2	n/a	5,018	Republic of Ireland and Northern Ireland	Murchie et al., 2007

A recent study in France showed that in 39% of *Salmonella* positive flocks at least one egg was positive as well; moreover these contained different serovars and molecular types that were the same as found in the corresponding contaminated flocks (Chemaly et al., 2009). Like contaminated yolk, soiled eggshells may easily contaminate food during food preparation.

The two UK studies used in the modelling work supporting this Scientific Opinion were carried out in the context of a wide-ranging study on the epidemiology and control of *Salmonella* in egg production (Reference: Department of Environment, Food and Rural Affairs: A monitoring, control and education package to assist the egg industry with *Salmonella* reduction and achieving EU targets. Final Report. DEFRA project reference OZO325[13]). The flocks for the individual egg study were selected on the basis of very high faecal and environmental contamination in the laying houses since high *Salmonella* prevalence eggs were needed in order to validate the pooling of eggs in batches of 40, as required by Community legislation. The flocks for the pooled egg study, except for one flock where various intervention trials were being carried out, were chosen from laying houses with minimal levels of positive screening samples, which would be unlikely to be detected as positive flocks by the monitoring specified in Community legislation. The sample is therefore biased in both directions. Details of the studies are available in the project report, available on request from the Veterinary Laboratories Agency (VLA) in UK.

Recent data on egg contamination from *S.* Enteritidis infected flocks was made available from a study carried out in Belgium (De Reu et al., unpublished[14]). Results obtained made available in the interim again report low occurrence *S.* Enteritidis positivity of eggs from infected flocks.

Survival, growth and inactivation of *Salmonella* in the egg and in/on the egg shell are influenced by series of factors and events during egg formation, after egg laying and collection, and during

13 EFSA kindly acknowledges Dr. Rob Davies from the UK Veterinary Laboratories Agency (VLA) and member of the *ad hoc* working group for sharing this information. Further details on this project are available on request to the VLA, UK.

14 EFSA wishes to acknowledge Dr De Reu for sharing data from his study to be considered in this EFSA Scientific Opinion.

processing and storage. A recent EFSA Opinion on cooling of eggs provides a detailed background on the survival, growth and inactivation of *Salmonella* in shell eggs (EFSA, 2008).

Salmonella can be deposited in different sites inside the egg (albumen, chalaziferous membranes, yolk) as a consequence of infection of hen's reproductive tissues. *Salmonella* contamination has been observed most commonly on the yolk membrane (Timoney et al., 1989; Shivaprasad et al., 1990; Gast and Holt, 2000a; Gast et al., 2002) or in the albumen (Humphrey *et al.*, 1989a; Mawer *et al.*, 1989; Gast and Beard, 1990; Shivaprasad *et al.*, 1990; Gast and Beard, 1992). Direct deposition inside the yolk is also possible but it is a rare event (ICMSF, 1996; Gast and Holt, 2001b; Gast et al., 2003). *S.* Enteritidis is the most commonly isolated serovar from egg contents, in accordance with the affinity to infect reproductive tissues which would contaminate eggs in the course of their formation and passage through the oviduct (Timoney et al., 1989; Boer and Wit, 2000). The LPS structure appears to be a key factor in oviduct persistence and survival in egg albumen.

Little is known about the number of cells in an internally contaminated egg at laying time, but it generally ranges from 1 to 400 *Salmonella* bacteria, with most eggs containing less than 40 *Salmonella* bacteria (Humphrey *et al.*, 1989a; Humphrey et al., 1991; Gast and Beard, 1992; Gast and Holt, 2000a; Chen *et al.*, 2002). No studies quantifying the number of *Salmonella* cells on the egg shell are available. Visually clean eggs may have *Salmonella* on the egg shell, but the numbers are necessarily low (Jones et al., 2004; Musgrove et al., 2005). When *Salmonella* is present on the shell surface, cells may penetrate the shell and associated membranes, which happens more easily immediately after lay or when the cuticle degrades (Bradshaw et al., 1990; Gast and Beard, 1990; Humphrey et al., 1991; Messens et al., 2006; Gast et al., 2006). No correlation has been found between eggshell and contents contamination from naturally contaminated eggs (Humphrey, 1994). During grading operations dirty and cracked eggs are separated, therefore occurrence of eggs with high counts of *Salmonella* or other bacteria on the egg shell decreases.

Albumen restricts microbial growth because of the bacteriostatic activity of several compounds. Yolk provides a rich-nutrient substrate supporting microbial growth (Humphrey and Whitehead, 1993; Braun and Fehlhaber, 1995; Kang et al., 2006; Chen et al., 2005; EFSA, 2009). Growth on the intact, dry egg shell is usually not observed because of low water activity and lack of suitable nutrients (Board, 1964; Brooks, 1960; Theron et al., 2003). Migration of *S.* Enteritidis in the albumen or through the yolk membrane to the yolk has been observed in experimental conditions using high incubation temperatures and high inoculum doses (Braun and Fehlhaber 1995; Hammack et al., 1993; Gast and Holt, 2000; Gast et al., 2007; Gast et al., 2008). Motility and chemotaxis influence how bacteria migrate inside the egg (Gast et al, 2007). It has also been shown that important *S.* Enteritidis multiplication can occur before membrane penetration on the vitelline membrane during the first 36 h of unrefrigerated storage (Gast et al., 2008). Differences in behaviour (growth rate, motility inside egg contents) between *Salmonella* strains have been reported (Gast et al., 2007).

Events such as water condensation or formation of cracks can be a risk factor facilitating *Salmonella* eggshell penetration (Messens et al., 2005). After shell penetration, *Salmonella* cells can grow in the egg contents. As the egg becomes older the extension and composition of the cuticle changes, and the eggshell is more easily infiltrated by bacteria (Nascimento et al., 1992; Messens et al., 2007). Eggs with low shell quality are more likely to be penetrated by *Salmonella* (Sauter and Petersen, 1974).

Egg contents alter with age: the albumen viscosity declines and the vitelline membrane degrades allowing the leaking of yolk contents and growth factors (particularly iron), facilitating *Salmonella* growth (Lock et al., 1992; Humphrey and Whitehead, 1993). Cells easily penetrate the vitelline membrane when the temperature is around 20ºC and its permeability increases with time at temperatures above 10ºC (Humphrey, 1994). When the egg is laid, the pH of the albumen ranges between 7.6-7.8, but it increases until 9.1-9.6 after three days of storage at ambient temperature due to the loss of carbon dioxide. This pH serves as a barrier since bacteria are progressively away from their optimum pH range.

Faecal contamination of the shell as a result of intestinal carriage may permit cross-contamination or trans-shell penetration into the egg contents (Humphrey, 1994). A variety of serovars including *S.* Enteritidis are routinely isolated from the egg shell (de Louvois, 1993b; Board and Kluwer, 1994; Humphrey, 1994; De Buck et al., 2004).

Cross-contamination along the food chain between surfaces and eggs can also lead to *Salmonella* contamination of the eggshell. Contamination in egg-packing plants may be a significant contributory factor to external contamination of eggshells (Davies and Breslin, 2003). This has been shown with sterilized eggs being processed in packing plants (De Reu et al. 2005) and the critical points are the candling, grading and packing area. Cross-contamination which involves other (raw or ready to eat) foods can occur also at home or in restaurants and catering sector. There is some evidence of cross-contamination as the cause of *Salmonella* egg-borne outbreaks (Thomas et al. 2006; Roberts Witteveen et al., 2009).

Overall, there is evidence indicating external contamination of the egg shells with different *Salmonella* serovars can occur during production and processing (e.g. cross contamination). There is however not sufficient data to quantitatively assess the public health risk related to consumer exposure due to *Salmonella* present on the egg shell. However, the public health impact of this pathway is considered smaller compared to transmission by eggs internally contaminated with *Salmonella Enteritidis*.

8. Estimation of the public health risk associated with different targets for *Salmonella* in flocks of laying hens in the EU

8.1. Description of currently available Quantitative Risk Assessment models

Several risk assessments have been undertaken examining this important pathogen-food combination.

In 1996, the US Food Safety and Inspection Service (FSIS) and the Food and Drug Administration began the development of a farm to table risk assessment model for *S.* Enteritidis in eggs and egg products. This was one of the first comprehensive microbiological risk assessments undertaken, and was published in 1998 (FSIS, 1998; Hope et al*.,* 2002). The baseline model estimated that among 69 billion eggs produced annually in the USA, 2.3 million would be contaminated with *S.* Enteritidis resulting in 661,633 human illnesses per year from consumption of these eggs. The contribution of *S.* Enteritidis from commercially pasteurized egg products was estimated to be negligible. Five mitigation scenarios were selected for comparison of their individual and combined effects on the number of human illnesses. Results suggest that mitigation in only one segment of the farm-to-table continuum will be less effective than several applied in different segments. For example, a policy that encourages quality assurance programs at the production level, cooling of eggs during processing and distribution, and proper food-handling techniques was likely to be more effective than a policy that only included one of these actions. The risk assessment informed the development of an Egg Safety Action plan in the US, which outlined a strategy to reduce the incidence of egg associated *S.* Enteritidis infections by 50% from 1998 to 2005 with the aim of elimination by 2010 (FSIS, 2005).

Whiting & Buchanan (1997) described the development of a quantitative risk assessment model for *S.* Enteritidis in pasteurised liquid egg. The model indicated that pasteurisation was appropriate to protect consumers even with a high incidence of flock infections and poor temperature control for eggs prior to breaking. However, risks were identified if pasteurisation was inadequate or there was temperature abuse during storage.

A subsequent risk assessment by FSIS, using newly available data and improved modelling techniques suggested that rapid cooling and pasteurization of shell eggs would be highly efficient control options (Schroeder et al., 2006).

The FAO/WHO have undertaken a risk assessment of *Salmonella* in eggs following requests from Member Countries and the Codex Alimentarius Commission (FAO/WHO, 2002). The assessment was intended to develop an example risk assessment framework model for world wide application, to assess currently available information and gaps and to use the risk assessment to explore risk management options and interventions. In this risk assessment, a reasonably large data set from reported outbreaks was analysed in order to estimate the probability of illness upon exposure to *Salmonella,* which provided a data-driven dose-response relationship. Although the outbreak data had uncertainties associated with them, and some of the outbreak data points required assumptions to be made, the model was based on real-world data, and was not subject to some of the flaws inherent in using purely experimental data. However, all data points were included in one set of parameter estimates, independently of *Salmonella* serovar and/or affected population. The dose-response relationship developed in this model has been used in other subsequent risk assessments.

A Finnish quantitative risk assessment investigated the risk of human infection due to *S.* Enteritidis in egg contents in Finland and attempts to assess the effect of interventions. It considers the whole breeding and production chain from the point of importation of layer grand parent chicks to calculating the number of human cases occurring in 2001, with and without certain interventions or changes in monitoring programmes. The number of human cases is based on data from 1999, when the infection risk was unusually high due to some breakdowns in laying flocks. The model allows for under-identification of infected flocks, resulting in a calculated prevalence of 0.3%, even though no flocks were found positive in 2001. Increasing the sampling frequency from three to four sampling rounds per flock cycle did not significantly reduce the number of human cases but failure to remove infected flocks, e. g. if for some reason they were not identified, would substantially increase the number of cases, especially if infection was present in imported breeding layer parent birds and the infected progeny of these were not all detected before entering the laying phase. Importation of contaminated table eggs would have the greatest impact on the occurrence of human cases, with numbers increasing proportionally in relation to the prevalence of contaminated eggs. This justified the maintenance of special import operations for eggs in Finland.

A quantitative risk assessment of *S*. Enteritidis in shell eggs has recently been conducted in Belgium (Grijspeerdt et al., 2005). The risk model was based on the FAO/WHO template for *S*. Enteritidis in eggs and egg products. Sensitivity analysis highlighted storage temperature of eggs as an important factor influencing the number of human *S*. Enteritidis infections.

Transmission of *S.* Enteritidis within flocks of laying hens determines the number of hens infected. This will in turn help to determine the number of contaminated eggs to be expected once *S.* Enteritidis is introduced into a flock. Moreover, parameters can be used in simulation and analytical mathematical models to determine the effect of intervention measures and surveillance studies.

Simulation models using relevant parameters pose an alternative to study the spread of a *Salmonella* infection in a flock. Van de Giessen et al. (1994) described a simulation model of the cumulative infection curve of *S*. Enteritidis in laying hen flocks, based on results from the Netherlands field situation at the time. The model was used to estimate the contribution of the different routes of infection into a flock. Main routes that were taken into account are: vertical and horizontal transmission and transmission via improperly cleaned and disinfected poultry houses. The authors concluded that there is a high probability of infection in the first part of the laying period originating mainly from the farm environment. Analysis of the cumulative infection curve of *S.* Enteritidis in laying flocks can be useful to evaluate intervention strategies.

Leslie (1996) presented a transmission model of *S.* Enteritidis in a battery laying flock of 10,000 birds over a 48 week egg production period, with five seeders. The results were compared to an experimental study in a broiler breeder flock kept on litter (Corkish et al., 1994). The five states of infection were: susceptible, infected, shedding, immune and carrier. The model parameters used were taken from the literature. The weekly number of contacts between hens, by direct contact and by

airborne transmission, made by one infectious case was assumed to be 2.14. The model indicated that 5% of the birds were likely to be culture positive for 18 weeks in three cycles of 7 weeks. This result was particularly sensitive to the contact rate that was used. This model can be used to study transmission rate, the implications for disease detection and the relative risks of egg contamination and its control.

Simulation models are convenient to assist the control of infectious disease, and are helpful for policy makers. The advantage is that they can demonstrate the effect of changes in parameter values on the outcome of the model, and they can be done without carrying out experiments or collecting field data, or for situations that cannot be examined experimentally or in the field if the pathogen is absent (Stegeman et al., accepted). The advantage may, however, be misleading as these models need to be based on solid data. If these data are not available, additional experiments or data analysis of outbreak data are essential (De Jong and Hagenaars, 2009). The number of studies providing parameter estimates for simulation models which consider *Salmonella* within flock dynamics is limited (see Thomas et al., 2009). Therefore, care should be taken to extrapolate these experimental results to large flocks of laying hens and under field conditions.

8.2. Estimation of the number of *Salmonella* Enteritidis contaminated eggs laid by flocks under different *Salmonella* flock prevalences

As discussed in the introduction section and elsewhere in this Opinion, the public health risk of *Salmonella* infections in laying hens is primarily associated with, and assumed to be proportional to, the production of eggs that are internally contaminated with *S.* Enteritidis. A quantitative risk assessment model was developed by EFSA's Assessment Methodology Unit (AMU) to support development of the BIOHAZ Opinion. The AMU Report is published as a stand alone document[15] but must be read as part of this Scientific Opinion in order to fully appreciate assumptions, uncertainties and data used in the modelling work.

The model presented in the AMU Report consists of two modules:

I. Estimation of the true prevalence of infection with *S.* Enteritidis in production flocks in a specific MS in a specific year, based on testing results as obtained in the official control programmes, both from operator testing and from official controls. This module considers the evolution of layer flocks as a function of time after entering the egg production environment in relation to three parameters: the probability of the flock being infected when entering the production environment (due to true or pseudo-vertical transmission, see EFSA (2009) and inadequate cleaning and disinfection of the production environment), the intensity of infection after housing and the recovery of infected flocks. The model also accounts for imperfect test sensitivity (i.e. the probability of detecting an infected flock is < 100%).

II. Estimation of the average numbers per million of internally and/or externally contaminated eggs with *S.* Enteritidis, in a truly infected laying flock. This module estimates the numbers per million of internally and/or externally contaminated eggs with *S.* Enteritidis produced by an infected flock, based on two recent empirical datasets from different production flocks in one MS, taking imperfect test sensitivity into account.

The number of contaminated eggs per million, modelled in the second module, is then multiplied by the true flock prevalence from the first module to arrive at an estimate of the total number per million

15 Scientific Report of EFSA on a Quantitative risk assessment of Salmonella Enteritidis in shell eggs in Europe. EFSA Journal 2010; 8(4):1588, available at: www.efsa.europa.eu/en/scdocs/scdoc/1588.htm

of internally and/or externally contaminated eggs in the specific MS and year. Uncertainties about both quantities are thus described by distributions. It is assumed that an infected flock is immediately removed from production at the time of testing (i.e. a possible delay due to time to complete testing, reporting and decision making is not included). Hence, the model primarily calculates the production of *S*. Enteritidis contaminated eggs from the expected flock prevalence over the production period taking into account the removal of the flock at the time of positive sample.

The model was based on previously published work from Finland on *S*. Enteritidis infection of flocks of laying hens and broiler chickens (EELA, 2003 and 2004), and was adapted to apply to different MSs. However, heterogeneity between production systems in MSs (e.g. housing types, vaccination, hygiene practices) is only incorporated in the model by inserting data that are specific to a given production system. For example, if from one MS the data represents results from vaccinated flocks, then the estimates are interpreted to represent vaccinated flocks in that production system in that MS. Likewise, if for some MSs it is not known whether the data represents vaccinated or non-vaccinated flocks, or if the data include results from both type of flocks, the result cannot be said to represent vaccinated or non-vaccinated flocks. Instead, the results would then represent only the MS-specific mix of vaccinated and non-vaccinated flocks. Therefore, one has to bear in mind what the data represents. Furthermore, within-flock infection dynamics over time during the laying period were not included at hen level. The model did allow analysing data from different testing times in the productive life of a flock to combine with age-specific overall detection sensitivity.

Model parameters are estimated using Bayesian statistical methods, using Markov Chain Monte Carlo simulation. The Bayesian approach implies that prior knowledge on all model parameters was to be specified, and this was based on expert knowledge in the working group.

After construction of the MS-specific baseline models, they were used to explore the impact of different scenarios reflecting different observed flock prevalence values and different test sensitivities. In each scenario, the actual flock sampling data of a MS is replaced by fictitious data, under which the whole estimation is rerun. The resulting estimates then describe what could be concluded if such data had been observed, under the given sampling scheme of that MS. These scenario analyses inform about the number of contaminated eggs per million under different prevalences (e.g. EU mean for *S.* Enteritidis, prevalence of *S.* Enteritidis in the MS reporting the maximum prevalence of both *S.* Enteritidis and *S.* Typhimurium, this is the targeted serovars) or target prevalences (i.e. 1% and 2%) and alternative testing strategies.

EFSA wishes to acknowledge those countries that submitted 2008 sample level detail data from their *Salmonella* control programmes in laying hens. Within the time frame of the project, data of sufficient sample level detail suitable for the model were available for only two MSs. Data used from flocks was based on *S.* Enteritidis only, and hence the prevalences reported here and in the report refer only to this serovar. In MS A, 325 flocks were tested at approximately 24, 39 and 54 weeks with 2, 1 and 4 positive flocks observed (observed prevalence 2.1%). In MS B, approximately 3,500 flocks were tested at 25, 40, 55 and 70 weeks with 4, 8, 5 and 7 positive flocks observed (observed prevalence 0.7%).

Three data sets were considered to estimate the number per million of *S.* Enteritidis internally and/or externally contaminated eggs from infected flocks. One older dataset from the USA (on *S.* Enteritidis internally contaminated eggs) was not included because the testing methods employed are now generally considered of low sensitivity, providing a low detection rate for positive eggs from known infected non-vaccinated flocks where the contamination rate would have been expected to be significantly higher. In addition, the flocks were unvaccinated (which does not concur with procedures for high prevalence MSs in the EU) and the strains of *S.* Enteritidis found in USA at that time were different to EU strains. Two recent datasets from the UK (on eggs contaminated internally and/or externally with *S.* Enteritidis) were made available to the working group (Reference: Department of Environment, Food and Rural Affairs: A monitoring, control and education package to

assist the egg industry with *Salmonella* reduction and achieving EU targets. Final Report. DEFRA project reference OZO325)[16]. One was based on the testing 100 pools of 40 whole eggs from flocks in which the prevalence was expected to be low and the other testing 180 to 300 individual whole eggs from 15 flocks in which the prevalence was expected to be high. Hence, none of the datasets were statistically randomised to avoid biases, and therefore results are presented using both individual datasets as well as using a combined dataset to illustrate the uncertainty in the model results due to these data restrictions. A further correction factor to estimate *S.* Enteritidis internally contaminated eggs only is discussed further down in this section.

At flock level, observed prevalences were 2.1% in MS A and 0.7% in MS B, while the true prevalence was estimated at 9.6% (median) with 4.5%-17.3% (95% CI) and 2.6% (median) with 1.7%-3.7% (95% CI) , respectively. Hence, the current EU control programme may not detect a large proportion of infected flocks if the estimates for the sensitivity of testing used in the model are correct. It is likely however that the most highly infected flocks that are most likely to be producing a significant proportion of contaminated eggs will be preferentially detected. Table 8 shows the results of estimating the *S*. Enteritidis contaminated eggs per million.

Table 8: Predicted number per million of internally and/or externally contaminated eggs with *S.* Enteritidis for MS Aand MS B hen data using the UK pooled and individual egg data with the lower and upper limits of the 95% credible interval (rounded up). Source: EFSA, 2010a.

Hen Data source	Egg Data source	*Salmonella* contaminated Eggs per million		
		Median	Lower cl	Upper cl
MS B	UKi	151.7	7.1	978.6
	UKp	13.9	0.2	132.2
	UKp-UKi	56.8	5.9	300.8
MS A	UKi	28.2	1.1	158.9
	UKp	2.3	0.0	20.7
	UKp-UKi	9.5	1.1	43.6

UKi=UK individual egg data; UKp=UK pooled egg data; cl=confidence limits

It can be concluded that there is considerable uncertainty in estimating the number per million of eggs contaminated with *S.* Enteritidis from the currently available data. In MS A, the number of eggs per million is estimated to range between 14 and 150 and in MS B between 2 and 28. Even wider ranges are suggested by taking the credible intervals of the estimates into account. The different estimates for these two MSs are related in part to differences in flock prevalence, but also in other, unobserved, differences related to production systems which cannot be included as data in the model (because they are unobserved), but as a whole contribute in different estimates for the model parameters, as can be seen from Table 9. Only more detailed stratifying of data could reveal underlying structures that may act behind the coarse single parameters describing the whole system. The Table shows that the initial probability of infection was fourfold higher in MS B compared to MS A, but that the intensity of infection after housing was up to seven times higher.

16 EFSA kindly acknowledges Dr. Rob Davies from the UK Veterinary Laboratories Agency (VLA) and member of the *ad hoc* working group for sharing this information. Further details on this project are available on request to the VLA, UK.

Table 9: Predicted values for the intensity of infection (lambda), intensity of recovery (mu) and initial infection probability (v). Source: EFSA, 2010a.

Hen Data	Parameter	Estimate	Lower	Upper
MS B	lambda (λ)[1]	0.068	0.010	0.172
	mu (μ)[1]	0.083	0.003	0.450
	v	0.017	0.001	0.051
MS A	lambda (λ)	0.010	0.003	0.019
	mu (μ)	0.093	0.003	0.480
	v	0.004	0.001	0.009

[1] lambda (λ) and mu (μ) are time-dependent variables. For further details please see EFSA, 2010a.

The model was then recalculated using different values of the observed prevalence, reflecting the current EU target (2%) and the proposed target (1%). However, as the EU target includes *S.* Typhimurium and/or *S.* Enteritidis these prevalence targets were also adjusted based on the average ratio of *S.* Enteritidis to (*S.* Enteritidis + *S.* Typhimurium) in the 2008 EU monitoring results. This resulted in prevalence targets for *S.* Enteritidis of 1.76 (instead of 2%) and 0.88% (instead of 1%), respectively. Furthermore, the model was recalculated for the EU-average observed flock prevalence for *S.* Enteritidis in 2008 (3.1%) and for the prevalence of *S.* Enteritidis in the MS reporting the maximum prevalence of both *S.* Enteritidis and *S.* Typhimurium (this is the targeted serovars). Finally, the effect of doubling the age-specific sensitivity from 0.3, 0.25, 0.35 to 0.6, 0.5, 0.7 was analysed.

Results of these scenario analyses are shown in Figure 4. The Figure shows that the relationship between observed flock prevalence and number per million of contaminated eggs with *S.* Enteritidis is approximately linear for all datasets that were analysed. In other words, decreasing the EU target from 2% to 1% observed flock prevalence will approximately reduce the number of *S.* Enteritidis internally and/or externally contaminated eggs on the market by 50%. However, the marginal effects of changing the target are relatively small.

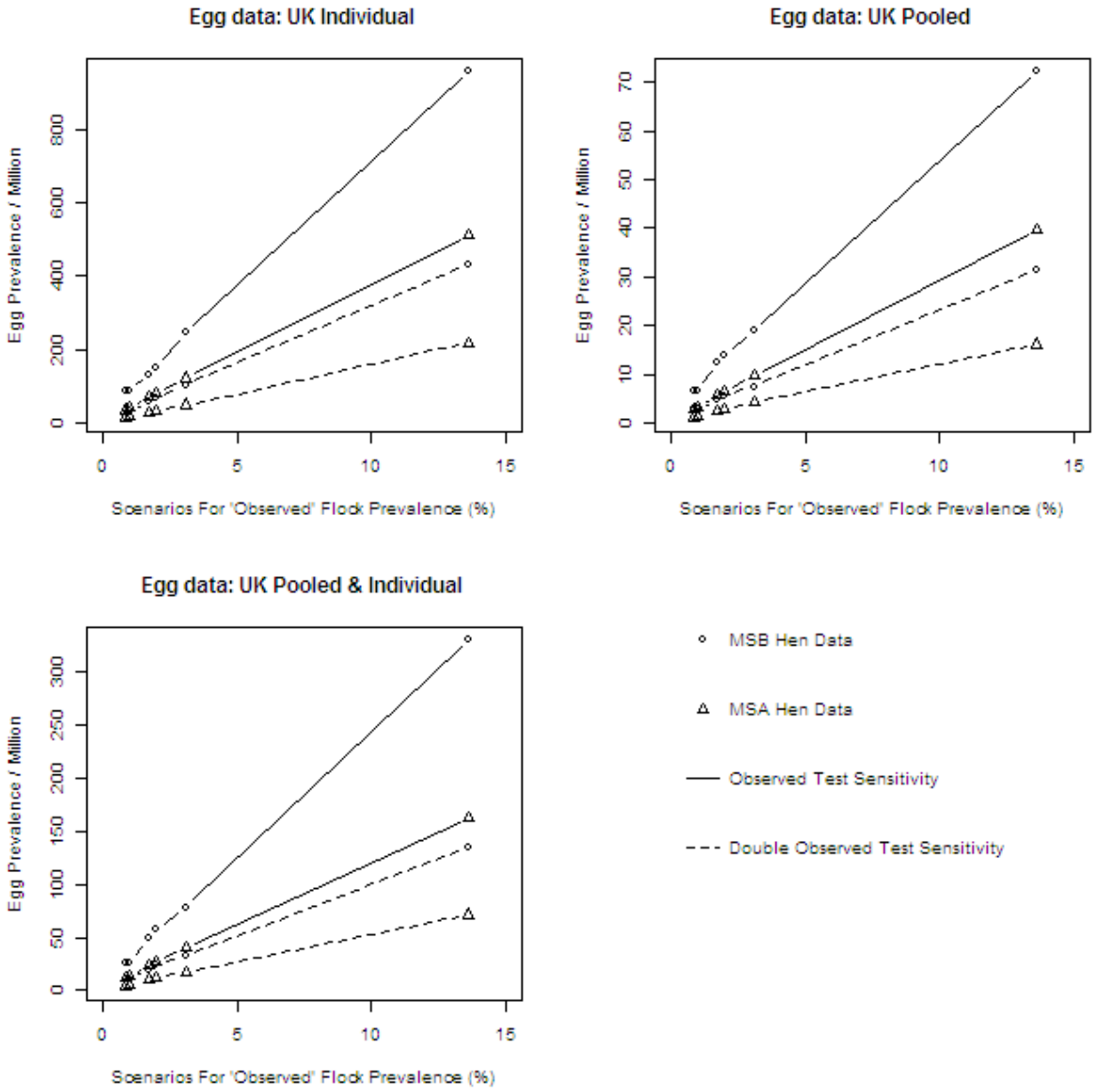


Figure 4: Predicted number of internally and/or externally contaminated eggs with *S.* Enteritidis per million for the different scenarios, depending on egg data (UK pooled or individual) and MS hen daata (MS A and MS B). Source: EFSA, 2010a.

It has to be noted that the slope of this relationship is different between MS A and MS B. This means that there is a different prediction of egg prevalence (i.e. number of *S.* Enteritidis contaminated eggs per million) at the same level of observed flock prevalence. This observation may be related to differences in production systems (e.g. housing, vaccination, hygiene practices), or the efficiency of detection of positive flocks.

Using hen data from MS B and combined egg data, the following predictions can be made based on the median estimates (see Table 10 for detailed results):

- The impact of changing from the prevalence of *S.* Enteritidis in the MS reporting the maximum prevalence of both *S.* Enteritidis and *S.* Typhimurium (this is the targeted serovars) in 2008 (13.6% for *S.* Enteritidis) to the EU target of 2% (worst case scenario, all *S.*

Enteritidis) would result in a reduction in the number of internally and/or externally contaminated eggs with *S.* Enteritidis from 330 to 57 per million, i.e. a reduction in the number of contaminated eggs of 273 per million.

- The impact of changing from the average EU target-related observed flock prevalence in 2008 (3.1% for *S.* Enteritidis) to the EU target of 2% (worst case scenario, all *S.* Enteritidis) would result in a reduction in the number of internally and/or externally contaminated eggs with *S.* Enteritidis from 79 to 57 per million, i.e. a reduction in the number of contaminated eggs of 22 per million.

- The impact by changing the EU target from 2% to 1% (worst case scenario, all *S.* Enteritidis) would result in a reduction in the number of internally and/or externally contaminated eggs with *S.* Enteritidis from 57 to 27 eggs per million, i.e. a reduction in the number of contaminated eggs of 30 per million.

Table 10: Predicted number eggs internally and/or externally contaminated with *S.* Enteritidis per million for MSB hen data using the UK pooled and individual egg data with the lower and upper limits of the 95% credible interval for different scenarios. Source: EFSA, 2010a.

	***S.* Enteritidis contaminated eggs per million**		
Scenario *S.* Enteritidis flock prevalence	**Median estimate**	**Lower**	**Upper**
Observed from the data	56.8	5.9	300.8
0.88%	26.6	2.1	161.3
1.00%	26.6	2.1	161.3
1.76%	48.7	5.0	261.7
2.00%	56.8	5.9	300.8
3.10%	78.3	8.6	401.1
13.60%	329.4	42.3	1509.1

The model used to support this scientific opinion suggests a linear relationship between the flock prevalence as currently observed in different MSs and the number per million of eggs contaminated with *S.* Enteritidis. The latter is assumed to be proportional to the public health risk. Based on the median estimates from the model, changing from the EU average flock prevalence reported in 2008 (3.1% for *S.* Enteritidis) to a transitional EU target of 2% is expected to result in approximately one third reduction in the number of *S.* Enteritidis contaminated eggs produced the EU. Changing the EU target from 2% to 1% of flocks remaining positive would result in a further reduction of a similar order of magnitude in the number of contaminated eggs produced in the EU. Moreover, it could also be concluded that the benefits that could be obtained by reducing flock prevalence in those MSs where observed prevalence remains higher than the current EU target would be higher than the benefit of changing the current EU target. These results are in line with the earlier WHO/FAO risk assessment on *Salmonella* in eggs and broiler chickens, where it was estimated that reducing flock prevalence would result in a directly proportional reduction in human health risk (FAO/WHO, 2002).

However, the absolute benefits of these reductions in flock prevalence are highly uncertain. There is a lack of data on the number of contaminated eggs produced by infected flocks, and on the true number of egg-related cases of human salmonellosis. For two MSs for which data in a suitable format for the model were available, there was a different prediction of egg prevalence at the same level of observed flock prevalence. This observation may be related to differences in production systems (e.g. housing, vaccination, hygiene practices), or the efficiency of detection of positive flocks.

In the scenario concerning observed prevalence, a similar benefit could be obtained by doubling the test sensitivity for flock monitoring samples because, with the given observed data, the estimates of initial infection probability and infection intensity would then be lower because the estimate of true prevalence would be lower, if the sensitivity was higher and eggs from infected flocks were removed from sale in a more timely way. The final number of eggs per million depends not only on sensitivity but naturally also on other model parameters, and their estimates would be updated in this scenario together with the sensitivity. The effect of higher test sensitivity is smaller if it is assumed that only it could be changed alone, keeping all other parameters as they were estimated from actual data. Therefore, greater reduction is achieved if both the prevalence is decreased and sensitivity increased. Thus, as the *Salmonella* monitoring defined in the EU Control Programme has limited sensitivity per sampling occasion, there may be considerable under-detection of infected flocks (see sections 4 and 6.2 of this Scientific Opinion). Additional public health benefits, similar to those considered to be achievable by changing the EU target from 2% to 1% flock prevalence, may be achieved by implementing controls based on more sensitive test protocols.

Since recent data was only available for whole eggs, (i.e. derived from culture of egg shells and contents together as a single pool) it is necessary to apply a correction factor to estimate the proportion of internally contaminated eggs. One earlier UK study (Davies & Breslin 2004), in which eggs were collected from known infected laying flocks, found a ratio of approximately 1:4 contents:shell contamination for *S.* Enteritidis. A wider range of additional serovars was found on shells than in contents and *S.* Typhimurium was not found in contents, although it was present alongside *S.* Enteritidis on one of the sampled farms. Other studies, (Wilson et al., 1998; Wall and Ward, 1999; FSA, 2004; Davies and Breslin, 2004; FSA, 2007; Murchie *et al.*, 2007), including an earlier unpublished UK study of eggs sampled in packing centres before the introduction of vaccination have suggested a smaller contents:shell ratio of approximately 1:10, which may reflect a longer period between laying of the eggs and sampling and testing than the UK farm study, in which testing was begun on the day after the eggs were laid, although a UK survey of eggs used in catering establishments carried out between 2005 and 2007 found a similar ratio of 1:5 (FSA, 2007). There is a high degree of uncertainty about these ratios because of the very low prevalence of positive eggs found in the various studies. An earlier UK study reported a ration 1:20 when examining non-UK produced shell eggs on retail sale in the North West of England and London (FSA, 2006).

In sum, it would be relevant to apply a correction factor of 0.1-0.25 to the results of the modelling exercise in order to more accurately assess the potential public health benefits of reduction in the flock prevalence targets. The proposed correction factor might still provide an underestimate of the number of eggs that are internally contaminated with *S.* Enteritidis, as there is normally a small proportion of externally contaminated eggs that may also be internally contaminated, especially in eggs from highly-infected flocks

Overall, the above results should be interpreted with care as different factors influencing the uncertainties around the estimates should be taken into consideration:

- Limitations regarding the data on flock prevalence of *S.* Enteritidis. As mentioned previously, data in the required format for the model was only obtained from two EU MSs. The results for other MSs, employing the same modelling approach, would be expected to be different from the results reported above, since the model attempts to be data driven and should be estimated for each MS data. Therefore, flock prevalences in these 2 MSs are not representative of other MSs where different distributions of production systems and flock *Salmonella* prevalence are found.

- Limitations regarding the data on egg contamination with *S.* Enteritidis. Data chosen for the modelling exercise is based on internal and/or external contamination of eggs with *S.* Enteritidis. Even though two data sources are used (i.e. single testing and batch testing), these data is from a single MS (i.e. UK).

- Model structure limitations. The model employed for this analysis does not take within-flock dynamics at hen level into account, but only indirectly via an age-specific sensitivity parameter. There is no detailed link between flock infection and egg prevalence over the life of a flock because the data relating to the detection of positive flocks per MS do not include egg samples or hen samples from the same named flocks. Parameter estimates therefore may be specific to the production systems from which the data were obtained i.e. results represent vaccinated flocks as the data were from vaccinated flocks, but the model would not necessarily describe unvaccinated flocks without having data specifically from those.

It has to be noted that several steps between the egg been laid in the farm and the table egg been placed on the market are not modelled (i.e. collection at the farm, transport to the classification and packaging centre, transport to market). A reduction between the number of eggs laid in the farm and the number of table eggs placed on the market is expected (e.g. eggs damaged or broken during handling in the different stages, eggs classified as 'Class B' eggs destined to heat treatment or animal by-products, surplus 'Class A' eggs destined to heat treatment). Nevertheless, it can be assumed that the number of table eggs internally contaminated with *S.* Enteritidis reaching the market will be equally proportional to the number of laid eggs internally contaminated with *S.* Enteritidis laid under the different *Salmonella* flock prevalences modelled.

The development of quantitative microbiological risk assessment models regarding *Salmonella* in laying hens that take into account within-flock dynamics for different production systems in order to aid in the development of improved *Salmonella* control programmes and the assessment of resulting public health impact that these may have should be recommended. Moreover, further experimental research and data collection on *Salmonella* dynamics in the laying hen flock and on production of contaminated eggs under field conditions should be encouraged in order to provide parameter estimates for these models.

8.3. Risk associated with pasteurised egg products

In the EU, both specific hygiene requirements for the elaboration of egg products and microbiological criteria for processes and end products are tools employed for the control and monitoring of the occurrence of *Salmonella* in egg products (Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin, and Regulation 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs).

Both Class A and Class B eggs can be sourced for the elaboration of egg products. Moreover and under a *Salmonella* risk point of view, it has to be pointed out that the sourcing of eggs from *S.* Enteritidis and/or *S.* Typhimurium positive flocks for egg products following treatment is a common practice from some member states (see Appendix X, and EFSA, 2010b). This is allowed under Community law (Reg. (EC) 1237/2007), which prescribes that the eggs have to be treated in a manner that guarantees the destruction of all *Salmonella* serovars with public health significance. The practical implementation of this regulation is subjecting the eggs or their contents to a pasteurisation treatment process.

Hygiene requirements for the handling of eggs and egg products are established by Regulation (EC) 853/2004. Section X of this regulation indicates in chapter II, point III, the hygiene requirements that must be applied for the sourcing of eggs for manufacturing of egg products and of the processes themselves.

For the case of egg products, hygiene requirements do neither prescribe specific treatments nor temperature/time combinations. Quoting the relevant section of the above mentioned regulation: '*After breaking, each particle of the egg product must undergo processing as quickly as possible to eliminate microbiological hazards or to reduce them to an acceptable level*'. In fact, other treatments

such as irradiation are applied to some egg components in certain MSs (EC, 2009) even though this is a rare practice.

Time/temperature parameters employed for the pasteurisation of egg products depend on the processed product (e.g. the heat treatment for the whites should be lower compared to yolks to avoid coagulation and loss of physical and technological capacities). Differences can be observed in these practices as the recommended time/temperature combinations in the EU appear to be higher than those in the USA (EUWEP, 2009[17]; Latimer et al., 2008).

Latimer et al. (2008) present a quantitative microbiological risk assessment for *Salmonella* in liquid egg products, including a module to estimate *Salmonella* reduction by pasteurisation processes. Table 11 shows the $\log_{10}$ reductions as presented in this paper from the USA. Similar estimates for the EU, based on the same model, suggest 8 to 9 $\log_{10}$–units reduction in whole eggs and yolks, and 9 to 12 $\log_{10}$–units reduction in extended shelf life products. Even though egg whites are pasteurised at lower temperatures than whole eggs or yolks, the predicted inactivation of *Salmonella* is much higher at 28 $\log_{10}$–units reduction as the pH of egg whites is much higher (8.8 vs. 7 for whole eggs and yolks).

Table 11: Comparison of predicted inactivation of *Salmonella* by pasteurisation processes for liquid egg products in the EU and USA employing a model developed by Latimer and colleagues (EUWEP, 2009; Latimer et al., 2008).

Product	EU		USA	
	Time/temperature (min/ °C) recommended[1]	**Log_{10} reduction**	**Time/temperature (min/ °C) [2]**	**Log_{10} reduction**
Whole egg	3.5-4/64	9.0-9.3	3.5/60	5.9
	2.5-3/68 (ESP[3])	11.7-12		
Yolk	6/64	8.0	3.5/61.1	5.5
	5-6/66 (ESP)	8.7-9.0		
Egg white	6/59	>12	3.5/56.7	3.3

[1] Source: EUWEP; [2] Source: Latimer et al., 2008; [3] ESP=Extended shelf life products; Results kindly provided by Dr Eric Ebel, U.S. Department of Agriculture, Food Safety and Inspection Service, Office of Public Health Science, Risk Assessment Division, Washington DC.

It must be noted that even though the predictions for EU conditions are based on the equations reported in Latimer et al. (2008), the parameters of those equations were estimated from experiments using temperatures that are below the temperatures recommended by EUWAP. Therefore, the predictions for EU conditions are necessarily less certain than those for USA conditions (i.e. the EU predictions are extrapolations beyond the experimental data).

Monitoring of the level of microbiological safety provided by a process or from a foodstuff is implemented in the EU following the bases of Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. This regulation defines a microbiological criterion as one that defines the acceptability of a product, a batch of foodstuffs or a process (EC, 2005). Depending on its intention and its time/point of application, there are two types of microbiological criteria defined in the EU: Process Hygiene Criterion and Food Safety Criterion.

As addressed in a previous EFSA Opinion (EFSA, 2007) an EU Process Hygiene Criterion gives guidance on, and is an indicator of, the acceptable functioning of HACCP-based manufacturing, handling and distribution processes. It sets indicative contamination values above which corrective

17 Information kindly provided by Mark Williams, Secretary General of the European Union association of Wholesale with Eggs, Egg Products and Poultry and Game (EUWAP).

actions are required in order to maintain the hygiene of the process in compliance with food law. An EU Food Safety Criterion defines the acceptability of food products. These criteria apply to the products placed on the market. If the criteria are not met the product/batch has to be withdrawn from the market.

For the case of egg products, both a Food Safety Criterion for *Salmonella* and a Process Hygiene Criterion for Enterobacteriaceae exist (see Table 12). There are also other limits for chemical compounds used as an index of the hygiene quality of the product (i.e. 3OH-butryric acid (10 mg/kg dry matter), lactic acid (1000 mg/kg dry matter). Nevertheless, it has to be highlighted that the testing frequencies are not laid down in EU legislation, and that these have to be based on HACCP and good manufacturing principles (EC, 2005).

Table 12: *Salmonella* related microbiological criteria applied to egg products (modified from Reg. (EC) No. 2073/2005).

Type of criteria	Food category	Micro-organisms	Sampling plan		Limits		Stage at which applies	Action in case of non-compliance
			n	c	*m*	*M*		
PHC[1]	Egg products	Enterobacteriacea	5	2	10 cfu/g or ml	100 cfu/g or ml	End of manufacturing process	Checks on the efficiency of the heat treatment and prevention of recontamination
FSC[2]	Egg products, excluding products where the manufacturing process or the composition of the product will eliminate the *Salmonella* risk	*Salmonella*	5	0	Absence in 25 g or ml	n/a	Products placed on the market during their shelf-life	Withdrawal from the market

[1] PHC = Process Hygiene Criteria; [2] FSC = Food Safety Criteria. n- number of units composing the sample; c- number of sample units given values between m and *M*

Reported compliance for microbiological food safety criteria for egg products for the period 2006 to 2008 can be found in Table 13. More non-compliance was reported in 2008 compared to the 2 previous years; 1.6% in 2008, and 0.4% and 0.2% in 2007 and 2006, respectively. This may be linked to the implementation of the *Salmonella* control programmes in laying hens, which might have implied that more eggs from *Salmonella* positive flocks were channelled to production of egg products (EFSA, 2010b). Alternative explanations of this increase in non-compliance could include recontamination and/or failures in process control. There is currently no data to conclude which cause is most likely.

Table 13: Compliance with *Salmonella* food safety criteria for egg products (Mainly data from official controls is presented). Source: EFSA, 2010b.

Year	Total single samples		Total batches	
	Number [a]	% non-compliant	Number [b]	% non-compliant
2008	2,744	1.6	5,323	0.3
2007	2,155	0.4	6,453	0.1
2006	4,063	0.2	288	0.7

a Sample comprises either 25 g of product or is not stated.
b Sample comprises either 25 or 25 ml of product.

It can be concluded that the diversion of eggs from flocks that are tested positive to the production of egg products subjected to heat treatment may lead to increased health risks, as pasteurisation may not be an absolute barrier to *Salmonella* contamination. Monitoring data at the EU level suggest that in

2008 there was a higher proportion of samples of egg products that did not conform with EU food safety criteria when compared to the previous two years.

Furthermore, methods employed in the validation of the performance of heat-processing treatments may overestimate the microbial load reduction achieved, as isolation of viable *Salmonella* in egg albumen after heat stress may be reduced. The validation of the capacity of industrial procedures for heat treatment of egg products in order to reduce the *Salmonella* risk is recommended.

It has to be mentioned here that *Salmonella* source attribution linked to consumption of egg and egg products is reported together by the EU MSs and presented under the same category in the Community Summary Report on trends and sources of zoonoses and zoonotic agents and food-borne outbreaks in the EU (EFSA, 2010b). In order to get better understanding on the relative significance of these two sources, outbreak reporting in the EU should differentiate between salmonellosis cases linked to table eggs and those linked to egg products.

8.4. Risk associated with meat from spent laying hens of *Gallus gallus*

Following a one year laying cycle, it is common that laying hen flocks are removed from production. Some flocks may be kept for a second laying cycle, following controlled moulting. It is interesting to note that the practice of moulting employing feeding restriction techniques has been epidemiologically linked to increased prevalence of *S.* Enteritidis in the revived flock (Holt *et al.*, 1994 and 1995; Gast and Ricke, 2003; Webster, 2003).

Under a regulatory point of view, Reg. (EC) 2160/2003[18] provides specific requirements concerning flocks of laying hens and fresh meat thereof. Precautions during slaughtering must be taken to reduce the spreading of zoonoses as far as possible, following Community legislation on food hygiene, but no explicit restriction on the use of that meat for human consumption does currently exist, either directly or after further processing. In fact, once a flock becomes positive to *Salmonella*, actions taken in the flock by MSs differ and this is sometimes irrespective of the serotype (see Table 3 in Appendix C). Details on fate of spent hens after the laying phase and available EU quantitative data on some production indicators is available in Appendix D.

Data on spent hen meat (or meat products) consumption in MSs would be of key relevance when assessing potential human exposure to *Salmonella* through these food commodities. These detailed data are not readily available as most of the reported data on poultry meat consumption relate to 'poultry' in general, where no inference in the proportion of hen meat consumed can be made (Magdelaine *et al*., 2008).

Further consideration has to be given to the final destiny of the flocks following a positive test to *Salmonella* or to *S.* Enteritidis and/or *S.* Typhimurium. Regulation (EC) No.1237/2007 allows for the slaughter of those flocks following steps to reduce the risk of spreading zoonoses as far as possible. Some EU MSs do implement measures in accordance with their national control programmes aiming at further reducing (or even eliminating) the risk. Thus, measures in some MSs include the destruction of the flock (i.e. not for human consumption) or application of heat treatment processes following slaughter or even full destruction and disposal not for human consumption (see Table 3, Appendix B, taken from EFSA 2010b). Nevertheless, some MSs report that flocks may still be sent for slaughtering with some special provisions to reduce *Salmonella* risk in the resulting product but without further heat treatment (e.g. logistic slaughter, slaughtering at the end of the working day), as seen Table 3, Appendix B. This should be cautiously interpreted as some MSs do not update the information collected in this table every year, and thus this practice may be less frequent or not in use any more.

18 As amended by Reg. (EC) 1236/2007 as regards the placing on the market of eggs from *Salmonella* infected flocks of laying hens.

Similarly to broiler meat, meat produced from 'spent hens' can be contaminated with *Salmonella*. The slaughtering and further processing of 'spent hens' implies several opportunities for the hen carcass and meat thereof to become contaminated with *Salmonella* from different sources including the exterior of the bird, the gastrointestinal contents and the slaughtering environment. *Salmonella* contamination in meat originated from *Salmonella*-free broiler flocks has also been associated to cross contamination during handling and processing, an extrapolation that is plausible for the processing of 'spent hens' (Corry *et al.*, 2002; Olsen *et al.*, 2003; Heyndrickx *et al*., 2002; Rasschaert *et al.*, 2007). There are no sufficient data to quantify the rate of this contamination in spent hen meat, or whether these would be higher or lower than for other *Gallus gallus* meat categories (e.g. broiler meat).

However, there is some indication that, due to several factors as flock age, immune-compromised stage at the end of lay, extra-intestinal infection and processing limitations (e.g. increased cross-contamination during slaughter due to technical limitations when using processing premises for broiler flocks) a higher prevalence of *Salmonella* contaminated spent hen meat could result when compared to broiler meat. These data are currently not reported separately (i.e. both broiler meat and spent hen meat reported together). However and as a reference, it could be stated that in Belgium in 2008 a total of 91 *Salmonella* positive batches out of 200 of spent hens were included in the reporting of *Salmonella* in broiler flocks (CSR, 2010) out of a total of 342 *Salmonella* positive flocks (total of 8,148 flocks tested). Moreover and as discussed in point (b) of section 3.2., the EU average of reported *S.* Enteritidis positive flocks accounts for at least over 50% of all *Salmonella* positive flocks. When comparing reported average prevalence data for *Salmonella* in laying hen flocks and in broiler flocks in the EU for the last three years, both the overall *Salmonella* average and *S.* Enteritidis average are higher in laying hen flocks than in broiler flocks (CSR, 2010). Finally, it has to be said that currently reported source-attribution data collected in the EU on human salmonellosis cases linked to the consumption of poultry meat of *Gallus gallus* do not differentiate between meat from spent hens or from other *Gallus gallus* sources (e.g. broilers).

Salmonella microbiological criteria are established by Reg. (EC) 2073/2005 for certain meat and meat products, including both process hygiene criteria and food safety criteria. These criteria, apply to products placed on the market during their entire shelf-life. Following a positive result, the batch has to be withdrawn and disposed of as a by-product or processed in a way that eliminates the risk of *Salmonella.* The regulation does not prescribe harmonised sampling frequencies, which is left for the official authority of each MS to decide and establish. At the same time, the period between sampling at retail and availability of the typed analytical results can take as long as 10 days (EN/ISO 6579), a period of time that may already exceed chilled poultry shelf life and thus the availability of the batch in the market (Mulder, 1982).

When intended for human consumption, spent hen meat is mostly commercialised as an ingredient of heat treated foodstuffs but some EU MSs market fresh meat from spent hens. Overall, heat treated spent hen meat could be considered to have a reduced effect in the potential human exposure to *Salmonella* from spent hen meat as compared to fresh spent hen meat. Special consideration should be made to the use of meat from spent hens of *Salmonella* positive flocks and in particular if a further heat treatment is not applied to the meat. Nevertheless, this situation also applies to meat from broilers, which dominates the market of meat supply from *Gallus gallus*. An ongoing EFSA working group is carrying out a quantitative estimation of the public health impact of *Salmonella* in broiler flocks (*Gallus gallus*), which should provide further valuable information (Ref. EFSA-Q-2008-293).

In conclusion, when intended for human consumption, meat from spent hens is mostly heat-processed but some MSs market fresh meat from spent hens. Currently, there are insufficient data to quantitatively evaluate the risk associated with human consumption of meat from spent hens when marketed as fresh meat. It is anticipated that the prevalence of *Salmonella* (including *S.* Enteritidis) in the meat from these flocks might be higher than in meat from broiler flocks, in particular if sourced from *Salmonella*-positive laying hen flocks.

In order to get a better understanding of the true significance of the human salmonellosis risk associated with the consumption of *Salmonella* contaminated spent hen meat and meat products in the EU, both data on the prevalence of *Salmonella* in spent hen meat and surveillance and reporting of source-attribution data in the EU of human salmonellosis linked to consumption of spent hen meat and products thereof should be collected and reported separately from other *Gallus gallus* sources (e.g. broilers).

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Answer to the terms of reference:

- Public health risks of *Salmonella* infection in laying hens are associated with four different exposure pathways: internally contaminated table eggs, externally contaminated table eggs, egg products and meat from spent hens.

- In the EU, two serovars (*Salmonella* Enteritidis and *Salmonella* Typhimurium) are considered of paramount public health significance. Together, they account for approximately 80% of all human isolates to which typing was applied. Other serovars do not individually exceed 1%.

- Attribution models from two Member States and outbreak data from the EU suggest that in relation to eggs from *Gallus gallus*, *Salmonella* Enteritidis is by far the serovar most frequently associated with human illness. This is related to the ability of this serovar to persistently colonise the avian reproductive tract, resulting in internally contaminated eggs, as well as egg shell contamination.

- Even though other serovars of *Salmonella* can be transmitted by egg shell contamination, the public health impact of this pathway is considered smaller compared to transmission by eggs internally contaminated with *Salmonella* Enteritidis. Therefore, the expected public health benefit of including additional serovars other than *Salmonella* Enteritidis in EU-wide prevalence targets for laying hens is expected to be small at present.

- The quantitative risk assessment model used to support this Scientific Opinion suggests a linear relationship between the flock prevalence as currently observed in different Member States and the number per million of eggs contaminated with *Salmonella* Enteritidis. The latter is assumed to be proportional to the public health risk.

- Based on the median estimates from the model, changing from the EU average flock prevalence reported in 2008 (3.1% for *Salmonella* Enteritidis) to a transitional EU target of 2% is expected to result in an approximately one third reduction in the number of *Salmonella* Enteritidis contaminated eggs produced the EU. Changing the EU target from 2% to 1% of flocks remaining positive would result in a further reduction of a similar order of magnitude in the number of contaminated eggs produced in the EU.

- The absolute benefits of these reductions in flock prevalence are highly uncertain. There is a lack of data on the number of contaminated eggs produced by infected flocks, and on the true number of egg-related cases of human salmonellosis. For two Member States, for which data in a suitable format for the model were available, there was a different prediction of egg prevalence at the same level of observed flock prevalence. This observation may be related to differences in production systems (e.g. housing, vaccination, hygiene practices), or the efficiency of detection of positive flocks.

- Diversion of eggs from flocks that are tested positive to the production of egg products subjected to heat treatment may lead to increased health risks, as pasteurisation may not be an absolute barrier to *Salmonella* contamination. Monitoring data at the EU level suggest that in 2008 there was a higher proportion of samples of egg products that did not conform with EU food safety criteria when compared to the previous two years.

- There are insufficient data to quantitatively evaluate the risk associated with human consumption of meat from spent hens when marketed as fresh meat. It is anticipated that the prevalence of *Salmonella* (including *Salmonella* Enteritidis) in the meat from these flocks might be higher than in meat from broiler flocks, in particular if sourced from *Salmonella*-positive laying hen flocks.

General conclusions:

- *Salmonella* control programmes as used in different Member States have been accompanied by a concurrent decline in the reported incidence of human salmonellosis, in particular due to *Salmonella* Enteritidis, both in outbreaks and sporadic cases.

- Provisional annual reduction targets for *Salmonella* Enteritidis and *Salmonella* Typhimurium have been achieved in 2008 by 19 out of 23 Member States and Norway. Furthermore, 8 Member States plus Norway and Switzerland reported a flock prevalence of below 2% for these two serovars, which is the current transitional target.

- As the *Salmonella* monitoring defined in the EU Control Programme has limited sensitivity per sampling occasion, there may be considerable under-detection of infected flocks. Additional public health benefits, similar to those considered to be achievable by changing the EU target from 2% to 1% flock prevalence, may be achieved by implementing controls based on more sensitive test protocols.

- Infection with *Salmonella* Enteritidis is most readily detected in the 2-3 week period following transfer of birds to the laying house. A variable rise in excretion towards the end of lay can also enhance detection during late lay, especially if the flock has been moulted.

- The benefits that could be obtained by reducing flock prevalence in those Member States where observed prevalence remains higher than the current EU target would be higher than the benefit of changing the current EU target.

RECOMMENDATIONS

It is recommended:

- The establishment of harmonised active surveillance of human salmonellosis in all Member States, including efforts to quantify the level of under-ascertainment and underreporting, in order to improve the evaluation of the human health effects of interventions in flocks of laying hens.
- Outbreak reporting in the EU should differentiate between salmonellosis cases linked to table eggs and those linked to egg products.
- The application of more intensive sampling on laying hen farms than the standard official sampling where a link to human salmonellosis cases is to be investigated in order to increase the chances of detecting flock infection.
- Develop quantitative microbiological risk assessment models regarding *Salmonella* in laying hens that take into account within-flock dynamics for different production systems in order to aid in the development of improved *Salmonella* control programmes and the assessment of resulting public health impact that these may have.
- Carry out further experimental research and collect data on *Salmonella* dynamics in the laying hen flock and production of contaminated eggs under field conditions to provide parameter estimates for these models.
- Further analysis of data from harmonised monitoring and control programmes in 2009 for all Member States.
- Validation of the capacity of industrial procedures for heat treatment of egg products in order to reduce the *Salmonella* risk.

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. CALCULATIONS OF THE ESTIMATE ON TRUE INCIDENCE, BURDEN OF DISEASE AND COSTS OF HUMAN SALMONELLOSIS IN THE EU 27

Table 1 below shows the estimation of the true incidence based on reported rates and serosurveillance, the disease burden and the cost of illness for human salmonellosis in the EU 27.

Table 1: Estimation of the true incidence, burden and costs of human salmonellosis in the EU 27.

A. True incidence based on reported rates			**Source/calculation**
a	Reported rate of salmonellosis, 2008	26.4 per 100,000	CSR 2010
b	Total population EU-27, 01/01/2008	498,000,000	EUROSTAT[19]
c	Total reported cases EU-27, 2008	130,000	a x b
d	Underreporting factor	5-100	Expert estimated
e	Total cases EU-27, 2008	660,000-13,000,000	c x d
B. True incidence based on serosurveillance			**Source/calculation**
f	Incidence rate of sero-infection	0.24 per year	Simonsen et al., 2009
g	Incidence of sero-infection EU-27	120,000,000	f x b
h	Ratio of symptomatic to asymptomatic cases	1:100 - 1:500	Estimated
i	Total cases EU-27	1,200,000 - 6,000,000	g x h
C. Disease burden			**Source/calculation**
j	Burden of salmonellosis NL, 2006	1,600 DALYs	Haagsma et al., 2009
k	Total cases NL, 2006	43,000,000	Ibid.
l	Burden per case	0.04	j / k
m	Burden of salmonellosis, EU-27	24,000-490,000 DALYs	e x l
D. Cost of illness			**Source/calculation**
n	Cost-of-illness salmonellosis NL, 2006	11,000,000 €	Haagsma et al., 2009
o	Costs per case	250 €	n / k
p	Cost-of-illness EU-27	170,000,000-3,300,000,000 €	e x o

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B. SALMONELLA IN LAYING HEN FLOCKS DURING PRODUCTION REPORTED THROUGH EU NATIONAL CONTROL PROGRAMS BETWEEN 2005 AND 2007

Table 1: Table 1. EU MS Reported occurrence of *Salmonella* in laying hen flocks during production and serovar distribution between 2007 and 2008 (source: MSs data reported to the EFSA Zoonoses Unit).

EU MS	Year											
	2007				2006				2005			
	Units tested	S. Enteritids	S. Typhimurium	Other serovars, non-typeable, and unspecified	Units tested	S. Enteritids	S. Typhimurium	Other serovars, non-typeable, and unspecified	Units tested	S. Enteritids	S. Typhimurium	Other serovars, non-typeable, and unspecified
Austria	2,565	2.5	0.5	1.4	2,419	1.9	0.3	1.0	3,488	1.1	0.2	0.5
Belgium	378	2.9	0.5	2.6	676	0.0	0.0	4.9	666	0.0	0.0	6.0
Bulgaria	10	0.0	0.0	0.0	-	-	-	-	-	-	-	-
Cyprus	-	-	-	-	-	-	-	-	-	-	-	-
Czech Republic	426	23.7	0.2	1.4	92	0.0	0.0	0.0	81	0.0	0.0	0.0
Denmark	510	0.2	0.6	0.2	565	0.2	0.0	0.2	658	0.9	0.0	0.2
Finland	626	0.0	0.2	0.2	1,639	0.0	0.0	0.0	1,827	0.0	0.1	0.0
France	2,960	2.7	1.1	0.0	3,099	3.4	0.6	0.0	3,208	0.0	0.0	0.0
Germany	5,105	1.4	0.2	0.2	10,143	1.0	0.2	0.4	4,873	0.8	0.1	0.3
Greece	40	0.0	0.0	0.0	17	0.0	0.0	0.0	214	11.7	3.7	32.7
Hungary	-	-	-	-	-	-	-	-	-	-	-	-
Ireland	-	-	-	-	340	0.0	0.3	0.0	217	1.4	0.0	1.4
Italy	773	2.1	0.4	7.2	312	4.2	0.6	5.4	307	2.6	2.3	13.7
Latvia	102	17.6	1.0	2.0	11	36.4	0.0	0.0	-	-	-	-
Lithuania	-	-	-	-	-	-	-	-	13	38.5	0.0	7.7
Luxembourg	8	25.0	0.0	0.0	8	0.0	0.0	0.0	-	-	-	-
Netherlands	2,161	9.9	0.0	0.0	2,055	4.1	0.3	0.0	1,952	3.3	0.2	0.0
Norway	676	0.0	0.0	0.0	-	-	-	-	-	-	-	-
Poland	3,814	7.0	0.2	3.0	1,819	4.0	0.3	6.0	1,865	3.4	0.1	4.6
Portugal	1	0.0	0.0	0.0	-	-	-	-	-	-	-	-
Slovakia	1,032	1.7	0.0	1.0	1,150	1.9	0.0	0.3	-	-	-	-
Slovenia	179	5.0	0.0	1.1	165	0.6	0.0	1.2	107	5.6	0.0	0.9
Spain	771	10.6	1.2	15.3	1,125	11.9	1.2	18.0	485	46.2	5.4	21.6
Sweden	590	0.0	0.3	0.2	670	0.0	0.1	0.0	859	0.1	0.0	0.0
Switzerland	-	-	-	-	1,828	0.2	0.0	0.0	1,631	0.5	0.0	0.0
United Kingdom	-	-	-	-	-	-	-	-	-	-	-	-
EU TOTAL (average for only those reporting data)	22,727	5.6	0.3	1.8	28,133	3.7	0.2	2.0	22,451	6.8	0.7	5.3

C. *SALMONELLA* MONITORING PROGRAMMES IN LAYING HENS (*GALLUS GALLUS*) PRODUCING TABLE EGGS, 2008

Table 1: Countries running an approved monitoring and control programme[1] according to Regulation (EC) No 2160/2003 and meeting at least minimum sampling requirements set out by Regulation (EC) No 1168/2006[2]. Source: EFSA, 2010b.

MSs with approved surveillance programme (Decision 2007/848/EC)		All MSs except Malta	
Non-MSs with approved surveillance programmes (ESA Decision No 364/07/COL)		NO	
MSs with EU co-financing (Decision 2007/782/EC as amended by Decision 2008/920/EC)		20 MSs except DK, FI, IE, LT, MT, SI, SE,	
Countries with additional sampling (see Table SA_NEW Layers_Add)		AT, CZ, DK, EE, FR, LT, NL, PL, SK, UK	
Minimum requirement according to Regulation (EC) No 2160/2003 as amended by Regulation (EC) No 1168/2006			
Rearing period		**Production period[3]**	
Day old chicks	Dead chickens / destroyed chickens	Week 24 ± 2 weeks	Faecal samples
	Samples from the inside of the delivery boxes (internal lining/paper/crate material)	At least every 15th week thereafter	Faecal samples
2 weeks before moving	Faecal samples		
Diagnostic methods used			
ISO 6579 (2002)		AT, BE, BG, CZ, EE, GR, IT, NO, PL, SE, SI[4], SK, ES	
Modified ISO 6579 (2002)		LV	
ISO 6579 (2002) / Amendment 1:2007		FI, UK	
AFNOR NF 47 100 and 47 101		FR	
The method described in the O.I.E. manual, 5th ed., 2004		SI	
Buffered Peptone water		PT	
Various bacteriological		DK, LT, UK	
No information		CY, DE, HU, IE, LU, MT	

1. Non-MS (EFTA members) must apply the EU legislation according to Decision of the EEA Joint Committee No 101/2006.
2. Regulation (EC) 1168/2006 sets the community targets for the reduction of the prevalence of certain *Salmonella* types in laying hen flocks of *Gallus gallus* and setting the testing scheme to verify the achievement of the community targets for S. Enteritidis and S. Typhimurium.
3. Once a year, the competent authority sample one flock per holding comprising at least 1000 birds
4. ISO 6579(2002), Annex D:2007

Table 2: *Salmonella* monitoring programmes in laying hens (*Gallus gallus*) producing table eggs, 2008 - additional sampling. Source: EFSA, 2010b.

Day old chicks		**Rearing period**		**Production period**	
Type of sample					
Meconium	AT, EE, FR, PL, SK	Faecal samples[3]	CZ[1], DK[1, 2], LT, SK	Blood samples	NL[1]
		Dust samples	FR, UK[4]	Egg samples	DK[2]
		Blood samples	DK[1, 2], NL[1]	Faecal samples collected more frequently than every 15th week	DK, IE, LT, SK

1. Number of samples depend on flock size
2. All flocks are sampled
3. Four weeks before transfer
4. Additional dust samples taken by a large proportion of UK producers on a voluntary basis before start of lay

Table 3: Control measures taken in laying hens (Gallus gallus) producing table eggs in case of Salmonella infections, 2008. Source: EFSA, 2010b.

Control measures	Countries
Serovars covered	
All Serovars	AT, DK, FI, NO, LT, SE[1]
S. Enteritidis and *S.* Typhimurium	BG, CZ, EE, ES, FR[2], LV, NL, IE, PL, SK, SI, UK[9]
Restrictions on the flock	
Immediately following suspicion	BG, DK, EE, FR, IE, NO, NL, PL, SI, SE
Eggs covered by restrictions already on the basis of suspicion	DK, FR, IE, NO, NL, PL, SE, SI
Consequence for the flock	
Recovery or slaughter	
Slaughtered	ES, GR, IE, PL, SK
Flocks destroyed	LT
Sanitary slaughter	DK, FR
Destruction	CY, CZ, SE, SI
Slaughter or destruction	BG, EE
Sanitary slaughter or destruction	NO
Slaughter and heat treatment or destruction	FI, SI
Treatment with antibiotics	AT[3], CZ, PL, SI[3]
Consequence for the table eggs	
Destruction	BG, CY, EE, SE[4]
Heat treatment	AT, BE, CZ, DK, FI, FR, IE[5], LT, NL[5], SE[3]
Destruction or heat treatment	ES, NO, PL, SK, SI, UK
Other consequences	
Feedingstuffs are restricted (heat treatment or destruction)	DK, EE, NO, SI, SE
Disposal of manure restricted	EE, FI, FR, NO, PL, SK, SI, SE
Cleaning and disinfection	
Obligatory	BE, BG, EE, FR, FI, DK, IE, LT, LV, NO, NL, PL, SK, SI, SE
Negative bacteriological result required before restocking	BG, ES, FR, FI, IE, LV, NO, NL, DK, SI, SE
Requirement of an empty period	DK, EE (21 days), FR, NO (30 days)

Control measures	Countries
Further investigations	
Epidemiological investigation is always started	EE, ES, FR, FI, IE, NO, NL, SE, UK, SI
Feed suppliers are always included in the investigation	EE, FI, IE, NO, NL, SE, SI
Contact herds are included in the investigation	EE, FI, FR, IE, NO, NL, SE
Intensification of the examination of non-infected flocks on the same farm	DK, FI, FR, IE, NO, NL, SE
Vaccination	
Mandatory	HU
Recommended	AT[6], BE
Permitted	DK[7], BG, CZ, EE[10], ES[8], FR, LT, LV, SK, SI, UK
Prohibited	FI, NO, SE

Note: No measures are fixed in Directive 2003/99/EC

1. In Sweden, for invasive serovars and non-invasive serovars different control strategies may be applied
2. In France, during the rearing period, *S.* Typhimurium and *S.* Enteritidis are included. During the table egg production period in holdings placing their eggs on the marked via an egg packing centre, only *S.* Enteritidis is included until 60 weeks, and a last sampling is used to detect *S*. Typhimurium
3. Non-invasive *Salmonella*
4. Invasive *Salmonella*
5. Eggs are pasteurised until the flock is destroyed
6. In Austria, vaccination against *S*. Enteritidis recommended
7. In Denmark, no vaccination occur, as no vaccines have been approved by The Danish Veterinary and Food Administration
8. In Spain, only in rearing period
9. Voluntary operator monitoring in the United Kingdom in 2007. All isolations of *Salmonella* must be reported
10. In Estonia, vaccination against *Salmonella* could only be performed basing on the Veterinary and Food Board approval
11. Minimum control measures are set out in Regulation (EC) 2160/2003, annex II (D).

Member States of the European Union and other reporting countries in 2008

(a) Member States of the European Union, 2008 (Name - Member State ISO Country Abbreviations 2008 Report)

Austria	AT
Belgium	BE
Bulgaria	BG
Cyprus	CY
Czech Republic	CZ*
Denmark	DK
Estonia	EE
Finland	FI
France	FR
Germany	DE
Greece	GR
Hungary	HU
Ireland	IE
Italy	IT
Latvia	LV
Lithuania	LT
Luxembourg	LU
Malta	MT
Netherlands	NL*
Poland	PL
Portugal	PT
Slovakia	SK
Slovenia	SI
Spain	ES
Romania	RO
Sweden	SE
United Kingdom	UK*

* In text, referred to as the Netherlands and the United Kingdom

(b) Non- Member States reporting in 2008 (Name - Country ISO Country Abbreviations 2008 Report)

Iceland	IS
Liechtenstein	LI
Norway	NO
Switzerland	CH

References Appendix C

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D. FATE OF SPENT LAYING HENS OF *GALLUS GALLUS* AFTER THE LAYING PHASE

End-of-lay hens (commonly known as 'spent hens' or 'boiling hens') become a sub-product or by-product of the egg industry, and face technological, environmental and animal welfare challenges (Broom, 1990; Kondaiah and Panda, 1992; Freeman *et al.* 2009).

It has been previously estimated that over 144 million live 'spent hens' must be removed annually from production worldwide (Lyons and Vandepopulier, 1996). Final destination of the 'spent hens' after finalising their laying productivity include:

- *Production of fresh poultry meat following routine slaughter practices*. The value of 'spent hen' fresh meat is limited due to its low yield and quality properties and the wide availability of reasonably priced broiler meat (Freeman *et al.*, 2009).

  'Spent hen' meat is mainly destined for further deboning processing (including mechanically recovered meat) followed by heat treatment. It is finally included in food stuffs such as canned soups, stews, pot-pies and dry soup mixes (Kondaiah and Panda, 1992; Gregory and Grandin, 2007).

  Fresh 'spent hen' meat doest not seem to be a widely marketed product in the EU. However, traditional *cuisine* from some southern European countries include the use of fresh 'spent hen' meat for some cooking recipes. On the other hand, fresh 'spent hen' meat in some EU countries seem to be a product that is mainly destined for exports outside the EU (Anonymous, 2006).

  Pet food production is another potential use of category 3 animal by-products originated from 'spent hen' slaughter, and of the meat produced if not destined for human consumption. Reg. (EC) 1774/2002 concerning animal by-products not intended for human consumption provides further details on the conditions of this practice.

- *Production of animal by-products following on farm euthanasia.* The on-farm killing and disposal of 'spent hens', in line with animal welfare regulations and the provisions of the Reg. (EC) 1774/2002, is another possibility. No detailed data on this practice in the EU have been found. However, recent discussion on technological aspects of this procedure can be found in literature (Freeman et al., 2009).

  Final destination usually includes rendering and/or incineration. Nevertheless, some Non-EU countries do still contemplate the use of rendered by-products of spent hens in the formulation of feed for farm animals, a practice not allowed in the EU following the animal protein feed-ban.

  Data on 'Spent hen' meat production in the EU for human consumption is scarce. Figures on spent hen slaughtering in the EU can be retrieved from EUROSTAT[20]. These data are presented in Table 1.

20 EUROSTAT: http://epp.eurostat.ec.europa.eu/portal/page/portal/eurostat/home/

Table 1: EUROSTAT data on number of slaughtered 'boiling hens'(end-of-lay hens) (x1,000 head) for reporting EU MSs. Period 2004 to 2008.

EU MS*	Year				
	2004	2005	2006	2007	2008
BG	n/a	n/a	n/a	1,472	1,057
DK	1,274	834	909	n/a	n/a
EE	n/a	n/a	n/a	n/a	23
FR	n/a	n/a	37,378	37,143	37,114
HR	4,100	4,476	4235	4400	n/a
HU	n/a	n/a	2002	924	1183
IT	n/a	n/a	n/a	n/a	23,152
LT	n/a	n/a	4,444	4,326	4,544
PL	18,220	49,668	42,967	62,216	55,287
PT	10	5,096	8	9	4910
SE	n/a	3,406	1,069	3,155	n/a
SI	485	365	397	298	290
SK	3,107	2,660	3,559	3,206	3,463
UK	36,514	37,752	36,592	37,232	38,412

* data shown only for MSs where data was available for at least one reporting year.
n/a=non available.

Nevertheless, data on spent hen population also retrieved from EUROSTAT show a marked different in reporting patterns and figures (see Table 2). Even though it is not possible to establish a link between population figures and slaughtering figures (*e.g.* uncertainty on the slaughtering date of the birds counted for in the population figures), underreporting on slaughtering numbers seems the most plausible explanation.

Table 2: EUROSTAT data on spent hen population (x1,000 head) for reporting EU MSs. Period 2004 to 2008.

EU MS*	Year				
	2004	2005	2006	2007	2008
AT	5,275	5,450	5,552	5,833	n/a
BE	12,157	10,613	10,508	9,598	n/a
BG	n/a	n/a	8,263	7,470	n/a
CY	n/a	550	472	517	n/a
CZ	11,112	9,917	n/a	10,661	n/a
DE	44,250	45,438	42,390	41,420	41308
DK	3,684	3,154	2,759	3,174	n/a
EE	897	797	n/a	626	n/a
ES	57,030	n/a	n/a	n/a	n/a
FI	3,210	3,180	n/a	n/a	n/a
FR	63,418	62,403	58,419	n/a	n/a
GR	14,224	13,823	12,779	13,021	n/a
HU	15,399	14,233	14,425	13,838	13,354
IE	3,691	3,954	4,133	1,640	n/a
IT	58,545	57,865	55,460	n/a	n/a
LT	4,219	4,377	4,386	4,386	n/a
LU	n/a	n/a	n/a	64,449	n/a
LV	n/a	2098	2115	2260	n/a
MT	415	477	500	564	560
PL	46,697	46,452	44,551	45,502	47,488
PT	8,516	7,742	7,677	7,871	n/a
RO	51,889	49,725	51,881	49,725	43,253
SE	4,995	5,065	n/a	n/a	n/a
SK	n/a	n/a	5,690	5,776	n/a
UK	48,073	38,000	36,560	n/a	n/a

* data shown only for MSs where data was available for at least one reporting year.
n/a=non available.

References Appendix D

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