European Food Safety Authority

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SCIENTIFIC OPINION

Quantitative estimation of the impact of setting a new target for the reduction of *Salmonella* in breeding hens of *Gallus gallus* [1]

Scientific Opinion of the Panel on Biological Hazards

(Question No EFSA-Q-2008-291)

Adopted on 26 March 2009

PANEL MEMBERS

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SUMMARY

Following a request from the European Commission, the Scientific Panel on Biological Hazards was asked to deliver a scientific opinion on a quantitative estimation of the impact of setting a new target for the reduction of *Salmonella* in breeding hens of *Gallus gallus*. More specifically, is asked to assess the relative impact on the prevalence of *Salmonella* in flocks of broilers and laying hens if a new target for reduction of *Salmonella* is set in breeding hens being 1% or less flocks remaining positive for all *Salmonella* serovars with public health significance, compared to (a) the theoretical prevalence at the end of the transitional period (1% of five serovars), and (b) the real prevalence in 2007 to be reported by the Member States. The *Salmonella* serovars with public health significance should be determined by the EFSA taking into account the criteria laid down in annex III to Regulation (EC) No 2160/2003.

The Scientific Panel on Biological Hazards highlighted that, as previously addressed, any *Salmonella* serovar that is not animal host-adapted is considered capable of causing gastro-intestinal illness of varying severity in humans, and thus should be considered of potential public health significance. Nevertheless, and when sufficient reliable data were available, the application of the criteria defined in the regulation that EFSA had to consider for determining the serovars with public health significance, allowed some relative categorisation of those

[1] For citation purposes: Scientific Opinion of the Panel on Biological Hazards on a request from European Commission on Quantitative estimation of the impact of setting a new target for the reduction of *Salmonella* in breeding hens of *Gallus gallus*. *The EFSA Journal* (2009) 1036, 1-68

serovars. *Salmonella* Enteritidis and *Salmonella* Typhimurium are responsible for the majority of reported cases of human illness and are considered as of paramount public health significance. All other serovars individually constitute less than 1% of reported human cases. Furthermore, *Salmonella* Enteritidis is the serovar most frequently associated with illness related to broilers and broiler meat, as well as with eggs and egg products. These, as well as other invasive serovars (*e.g.* *Salmonella* Dublin, *Salmonella* Virchow, *Salmonella* Heidelberg and *Salmonella* Choleraesuis), are associated with serious human illness and increased mortality. Antimicrobial resistance is particularly associated with *Salmonella* Typhimurium, but also with several other serovars including *Salmonella* Enteritidis, *Salmonella* Paratyphi-B, *Salmonella* Hadar, *Salmonella* Virchow, *Salmonella* Heidelberg, *Salmonella* Newport and *Salmonella* Infantis.

The Scientific Panel on Biological Hazards concluded that *Salmonella* Enteritidis and *Salmonella* Typhimurium have the greatest potential for vertical and pseudo-vertical transmission, from breeding hens to their progeny in the broiler meat and egg layer chains. EU-control measures for these two serovars in breeding hens are expected to contribute to the control of *Salmonella* infections in production stock, and to reduce human health risks from poultry. The marginal benefits of additional EU-wide control for other serovars in breeders (including the currently regulated serovars *Salmonella* Hadar, *Salmonella* Infantis and *Salmonella* Virchow) are relatively small: they are less frequently associated with human illness and have less potential for vertical transmission (in particular for laying hens, as well as minimal relevance in terms of contamination of table eggs). Biosecurity measures applied to control *Salmonella* Enteritidis and *Salmonella* Typhimurium would also have a beneficial effect to control horizontal transmission of other serovars by contaminated feed, resident contamination in hatcheries and farms and spread of infection by movement of personnel, wild animals, equipment and other fomites.

Harmonised monitoring and reporting of *Salmonella* occurrence in different poultry populations is still largely incomplete in the EU. Consequently, there is currently insufficient data to quantify the impact of controlling *Salmonella* prevalence in breeders on the prevalence in production stock. Available risk assessment models are restricted to two EU Member States, and refer to earlier situations, in which different control measures were implemented. There are indications that for those serovars, for which vertical transmission is possible, controlling *Salmonella* prevalence to very low levels is necessary to achieve a low prevalence in production stock.

The Scientific Panel on Biological Hazards recommends that EU-wide targets for serovars other than *Salmonella* Enteritidis and *Salmonella* Typhimurium in flocks of breeding hens should be tailored to the particular situation in each Member State. At the same time, it is recommended that a further evaluation and quantification of the relationship between breeding and production flocks be carried out when more harmonized data from control programmes in each sector are available. Such considerations should include the further development of quantitative risk assessment models, taking data for specific serovars into account.

Key words: *Salmonella*, poultry, breeding hen, *Gallus gallus*, microbiological target.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

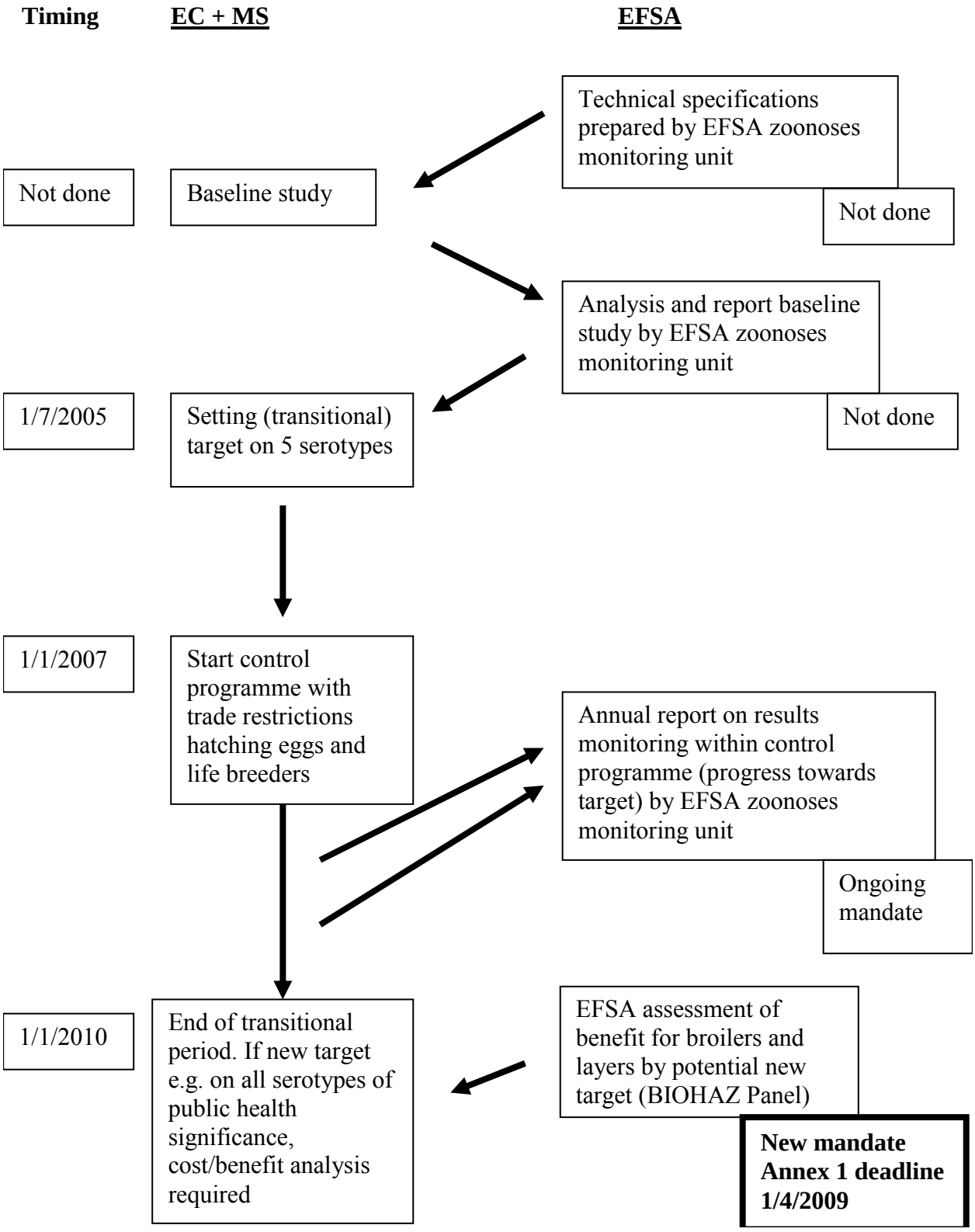
The presence of *Salmonella* in poultry populations is considered as a risk factor for the presence of *Salmonella* in meat and eggs. Targets are being set for the reduction of certain *Salmonella* serovars in different poultry populations within the frame of Regulation (EC) No 2160/2003[2] on the control of zoonoses. As a transitional measure, a limited number of serovars have been considered for reduction during the first three years of the control programme. Before the end of this period, a review of the serovars should be considered.

As regards breeding hens of *GaIlus gallus,* Regulation (EC) No 1003/2005[3] transitionally sets a target for reduction being 1% or less flocks remaining positive for *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Salmonella* Hadar, *Salmonella* Infantis or *Salmonella* Virchow by the end of 2009. This Regulation also harmonises the monitoring in breeding hens in all Member States since the beginning of 2007. Therefore, comparable prevalence data of all Member States are available. These prevalence data are forwarded by Member States to EFSA's Zoonoses Data Collection unit.

Before a new target is considered for reduction of *Salmonella* beyond 2009, a cost/benefit analysis must be carried out. Although the ultimate benefit is the public health impact of a possible new target, a step by step approach can be considered because of the complexity of the analysis. A first step is the assessment by the EFSA of the benefit of a new target in breeding hens on the relative prevalence of *Salmonella* in flocks of broilers and laying hens as indicated in the flow chart included in the next page.

[2] OJ L 325, 12.12.2003, p. 1. Regulation as last amended y Commission Regulation (EC) No 1237/2005
[3] OJ L 170, 1.7.2005, p. 12

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



TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

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

- the theoretical prevalence at the end of the transitional period (1% of five serovars), and
- the real prevalence in 2007 to be reported by the Member States.

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

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group on "a quantitative estimation of the impact of setting a new target for the reduction of *Salmonella* in breeding hens of *Gallus gallus*" for the preparation of this opinion: Mariann Chriél, Pierre Colin, Robert Davies, Arie Havelaar (Chair), Bela Nagy, Antonia Ricci and Ekelijn Thomas.

Assistance to the working group from Juan Carrique-Mas and Andy Wales is also acknowledged, in particular for their contribution to the annex included as part of this mandate (Annex on the analysis of *Salmonella* monitoring and prevalence figures in poultry (*Gallus gallus*) in the European Union between 2004-2007). Further acknowledgement is given to Wilfrid van Pelt and Tine Hald for providing data included in Table 3 of this Opinion.

At the same time, the following EU organisations are acknowledged for their availability when providing data sources on poultry movements within the EU: The Association of Poultry Processors and Poultry Trade in the EU countries (AVEC), the European Egg Packers and Traders Association (EEPTA) and the European Union of Wholesale with Eggs, Egg Products and Poultry and Game (EUWEP).

ASSESSMENT

1. Introduction

Directive 2003/99/CE on the monitoring of zoonoses and zoonotic agents, which repeals the previous Council Directive 92/117/EEC, has the purpose of ensuring that zoonoses, zoonotic agents and related antimicrobial resistance are properly monitored, and that foodborne outbreaks receive proper epidemiological investigation. These objectives will enable the collection in the Community of the information necessary to evaluate relevant trends and sources.

Regulation (EC) 2160/2003 on the control of *Salmonella* and other specified foodborne zoonotic agents, which entered into force on the 12th of December 2003[4], foresaw the establishment of Community targets for the reduction of prevalence of *Salmonella* serovars with public health significance in different animal populations. At the same time, it also established the minimum sampling requirements necessary for the monitoring of the prevalence of *Salmonella* following the implementation of the national monitoring and control programs.

The first Community target that had to be established 18th months after the date of entry into force of the regulation, was for *Salmonella* serovars with public health significance in populations of breeding flocks of *Gallus gallus*. Regulation (EC) 1003/2003 set that target to be the reduction, by 31 December 2009, of the maximum percentage of adult breeding flocks comprising at least 250 birds remaining positive to 1 % or less to the following *Salmonella* serovars: *S.* Enteritidis, *S.* Typhimurium, *S.* Infantis, *S.* Virchow and *S.* Hadar. Moreover, this regulation also established the testing scheme necessary to verify the achievement of the Community target.

The numerical establishment of the target for *Salmonella* prevalence in breeding flocks of *Gallus gallus* was based on the Report on results of monitoring / control of *Salmonella* in breeding flocks of *Gallus gallus* in the European Union and Norway in 2004 (EC, 2005a). The data presented in that report were collected through the implementation of the now repealed Council Directive 92/117/EEC, which contained in its Annex III detailed compulsory requirements for the regular testing of breeding flocks of *Gallus gallus*.

As a transitional measure, five *Salmonella* serovars were selected based on the ranking of the frequency of the serovars isolated from cases of human salmonellosis in the European Union in 2000, 2001 and 2002. These data were taken from the EC reports on Trends and sources of zoonotic agents in animals, feedingstuffs, food and man in the European Union (7 Member States (MSs)) and Norway in (EC, 2003; EC, 2004a) (See Table 1 and Figure 1).

[4] And applied 6 months after this date, this is 12th June 2004.

Table 1. The most frequent *Salmonella* serovars isolated from human salmonellosis in nine countries[1], as in 2000 and 2001 (EC, 2003)

Rank	2000	%	2001	%
1	*S.* Enteritidis	59,14	*S.* Enteritidis	71,49
2	*S.* Typhimurium	13,03	*S.* Typhimurium	16,19
3	*S.* Hadar	1,77	*S.* Virchow	1,90
4	*S.* Virchow	1,36	*S.* Hadar	1,62
5	*S.* Infantis	0,87	*S.* Infantis	1,05
6	*S.* Agona	0,75	*S.* Agona	0,95
7	*S.* Brandenburg	0,68	*S.* Newport	0,80
8	*S.* Newport	0,53	*S.* Braenderup	0,72
9	*S.* Blockley	0,46	*S.* Bovismorbificans	0,57
11	*S.* Braenderup	0,43	*S.* Brandenburg	0,54
12	*S.* Stanley	0,39	*S.* Derby	0,49
13	*S.* Derby	0,36	*S.* Paratyphi B, var. Java	0,48
14	*S.* Montevideo	0,31	*S.* Stanley	0,33
15	*S.* Paratyphi B, var. Java	0,31	*S.* Livingstone	0,27
16	*S.* Bredeney	0,25	*S.* Blockley	0,23
17	*S.* Thompson	0,14	*S.* Goldcoast	0,23
18	*S.* Saint-Paul	0,07	*S.* Oranienburg	0,12
19	*S.* Senftenberg	0,07	*S.* Dublin	0,10

[1] The ranking is summarised from Austria, Belgium, Denmark, Finland, Ireland (NRL), Norway, Sweden (domestic) and UK (England, Wales and Northern Ireland)

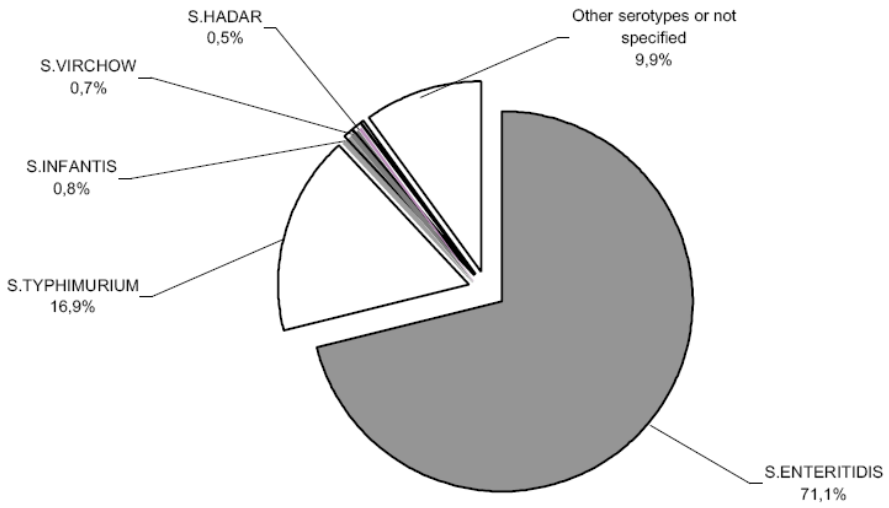


Figure 1. Distribution of the top five *Salmonella* serovars in human salmonellosis in the EU in 2002 (EC, 2004a)

2. Public Health significance of *Salmonella*

2.1. Reported and true incidence of salmonellosis in the EU

In the EU, human salmonellosis cases have been reported via Basic Surveillance Network (BSN) in the period 2000-2005. In 2006, however, in order to improve collection, validation, storage and dissemination of surveillance data from the MSs one integrated European surveillance system (TESSy) was implemented. TESSy covers all statutory communicable diseases with the appropriate level of detail according to their priority. At the same time, the Enter-net, international surveillance network for gastrointestinal diseases that replaced the Salm-Net surveillance network in 1997, has been integrated into the activities of the European Centre for Disease Prevention and Control (ECDC). Thus, data for 2007 and onwards on confirmed cases of human salmonellosis in the EU will primarily be reported via TESSy.

The number of confirmed human salmonellosis cases reported in the EU since 2004 is presented since 2005 in the Community Summary Report (CSR) on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks issued by EFSA (EFSA, 2005a; EFSA, 2006; EFSA, 2007; EFSA, 2009a). Those cases reported between 2001 and 2003, are previously presented in the European Commission report on Trends and sources of zoonotic agents in animals, feedingstuffs, food and man (EC, 2003; EC, 2004a; EC, 2005b).

Salmonellosis is the second most commonly reported food borne zoonoses in the EU as a whole. In 2007, a total of 155,540 confirmed cases of human salmonellosis were reported via The European Surveillance System (TESSy) from 30 countries including 27 EU Member States (MSs) and three non-MSs, and directly to EFSA from one country (Switzerland) (EFSA, 2009a). The number of confirmed human salmonellosis cases in the EU reported, first via BSN (Basic Surveillance Network) and from 2006 via TESSy, has decreased since 2005; from 173,879 (or 38.2 / 100,000) confirmed cases in 2005 to 164,011 (or 35.8 / 100,000) in 2006, and to 151,995 (or 31.1 / 100,000) in 2007. This represents a 7.3% decrease from 2006, despite contributions from countries that became EU members in 2007 (Bulgaria and Romania), and a 12.6% decrease from 2005 in EU MSs. Overall, total case counts of salmonellosis have decreased since 2004.

The decreasing Community trend since 2004 is statistically significant. However, the Community trend may not reflect the situation in a group of MS or in individual MS. For example, there is no significant trend in the old EU-15[5], where control programmes for salmonellosis generally have been implemented for a longer time than in new MS[6]. At the MS-level, despite Germany reporting 2,825 more confirmed salmonellosis cases than in 2006, the total number of confirmed cases within the EU decreased between 2007 and 2006, largely due to the Czech Republic reporting 6,531 fewer cases and Hungary reporting 2,814 fewer cases compared to 2006, respectively. Of the 27 MSs, 15 (60.0%) reported a decrease in *Salmonella* notification rates in 2007, while eight (32.0%) experienced an increase in notification rates compared to the previous year. The different sensitivities of MS reporting systems may have influenced these figures. Consequently, results are generally not directly comparable between MS and sometimes not even between years in one MS. The reported rates may be affected by large outbreaks, which increasingly involve products of other than

[5] No data for Luxembourg reported.
[6] Information kindly provided by the European Centre for Disease Control.

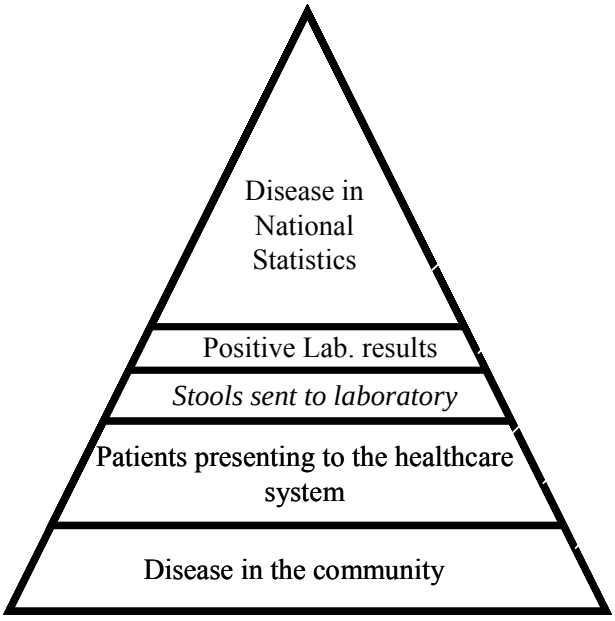
animal origin, such as salad vegetables, sprouted seeds and spice products (EC, 2002; Ezenna, 2009).

As previously addressed by EFSA in its Scientific Opinion on a quantitative microbiological risk assessment on *Salmonella* in meat (EFSA, 2008a), most ongoing surveillance schemes for foodborne disease in humans depend upon symptomatic patients consulting with, or presenting to, a primary care physician. Without this step the illness is unlikely to be recorded in any official statistics. The loss of data at various points along the surveillance chain from patient, through laboratory tests, to official statistics is generally represented by a pyramid (Figure 2, taken from EFSA, 2008a). Disease in the community forms the base of the pyramid while those cases that reach official statistics form the apex.

There have been relatively few attempts to calibrate human *Salmonella* surveillance data at national surveillance institutes, but some researchers have attempted to equate disease in the population to what appears in official statistics. In a three year study of infectious intestinal disease (IID) in England in the mid 1990s the investigators determined that for every laboratory-confirmed case of *Salmonella* reported to national surveillance, 3.2 cases occurred in the community (Wheeler *et al.*, 1999). This means that national statistics on laboratory-confirmed salmonellosis in England should be multiplied by 3.8 in order to describe better the community burden of salmonellosis. There are few similar examples from other countries. In the Netherlands, the value by which it should be multiplied is approximately 13.4 (calculation based on Kreijl *et al.*, 2006) whilst in the US it was estimated to be 38.6 (Voetsch *et al.*, 2004). Surveillance systems "eavesdrop" on the healthcare system, and their organisation in MS varies considerably. For example, the surveillance system in the UK is highly centralised whilst those in MS like Germany and Spain are highly federalised.

How differences in the organisation of surveillance might impact on reporting efficiency has not been investigated in a systematic way across the EU. A work package in the Med-Vet-Net Network of Excellence[7] aims to support priority setting of foodborne and zoonotic pathogens at the European level. One task involves the collection and evaluation of existing data on the incidence, health outcomes and costs of foodborne and zoonotic illness in 8 MSs. This task includes reconstruction of the surveillance pyramid for several enteric pathogens, including *Salmonella* spp. Data will come from telephone and internet surveys, complemented with expert estimates. It is likely that there is considerable variation in reporting efficiency across MSs.

[7] Further information available at: www.medvetnet.org



	England Based on IID*	Netherlands Based on Sensor**
Disease in National Statistics	1	1
Positive Lab. Test	1.2	NA
Stools to Lab.	*NA*	*NA*
Patients Presenting to Physician	2.3	4.9
Disease in the Community	3.2	13.4

*IID = Infectious Intestinal Disease Study; **Sensor & Netherlands Institute for Primary Health Care (NIVEL) Studies; NA = Not Available

Figure 2. Surveillance pyramid showing the multiplier or factor by which values should be multiplied, for *Salmonella* in England and the Netherlands (EFSA, 2008a).

2.2. *Salmonella* serovars of Public Health significance.

As previously addressed by EFSA, any serovar that is not animal host-adapted is considered capable of causing gastro-intestinal illness of varying severity in humans (EFSA, 2004a), and thus should be considered of public health significance. The relative frequency of serovars originating from poultry differs and dynamic changes occur between regions and production type. *S.* Enteritidis predominantly originates from layers or egg products, while *S.* Typhimurium originates from cattle, pigs and poultry in different proportions.

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 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 broiler meat and eggs originating from *Gallus gallus* will be discussed in the following section.

2.2.1. *Salmonella* serovars in human salmonellosis.

The ranking of the serovars most frequently isolated from cases of human salmonellosis cases in European countries for 2007 and 2006, as reported in the CSR, is presented in Table 2 (EFSA, 2009a).

Table 2. Distribution of the 10 most frequent *Salmonella* serovars from confirmed salmonellosis cases in humans. TESSy data, 2006 – 2007 (EFSA, 2009a)

2007			2006		
Serovar	**N**	**%**	**Serovar**	**N**	**%**
Enteritidis	81,472	64.5	Enteritidis	90,362	71.0
Typhimurium	20,781	16.5	Typhimurium	18,685	14.7
Infantis	1,310	1.0	Infantis	1,246	1.0
Virchow	1,068	0.8	Virchow	1,056	0.8
Newport	733	0.6	Newport	730	0.6
Stanley	589	0.5	Hadar	713	0.6
Hadar	479	0.4	Stanley	522	0.4
Derby	469	0.4	Derby	477	0.4
Kentucky	431	0.3	Agona	367	0.3
Agona	387	0.3	Kentucky	357	0.3
Other	18,562	14.7	Other	12,790	10.0
Total	**126,281**	100%	**Total**	**127,305**	100%
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, Slovakia, Slovenia, Spain, Sweden and the United Kingdom (England, Wales, Scotland and Northern Ireland)

The data in Table 2 show that in the EU-total in 2006-2007, *S*. Enteritidis and *S*. Typhimurium were associated with more than 80% of all reported cases of human salmonellosis where the isolate was typed. The other three serovars for which targets in breeding hens of *Gallus gallus* are set according to Regulation (EC) No 1003/2005 (*S*. Hadar, *S*. Infantis and *S*. Virchow) together were associated with approximately 2% of cases and *S*. Hadar is no longer amongst the 'top 5' serovars. 10-15% of cases were associated with a variety of other serovars, none of them individually exceeding 1%. On the other hand, 9,814 and 17,359 *Salmonella* isolates in 2006 and 2007 respectively, were "unknown", which includes untyped isolates (no typing was attempted) and untypeable isolates (typing was attempted but outcome was not successful).

Source attribution information on serovars associated with *Gallus gallus* is available only in a few MS. Data based on microbial subtyping (see EFSA 2008b for details on microbial subtyping) from the Netherlands and Denmark as presented in Table 3.

Table 3. Proportion of cases and serovar distribution of human salmonellosis attributed to *Gallus gallus* based on microbial subtyping (Raw data kindly supplied by Wilfrid van Pelt (RIVM, Bilthoven, The Netherlands) and Tine Hald (FOOD-DTU, Soborg, Denmark)).

Country	The Netherlands		Denmark		
Period	2000-2008		2003-2007		
Reservoir/vector	Broilers/ broiler meat	Layers	Broilers National	Broiler meat, imported	Layers
Attributable fraction (all serovars)	12.2%	32.5%	3.2%	11.8%	12.3%
S. Enteritidis	6.9%	26.9%	0.2%	6.0%	11.2%
S. Typhimurium	1.1%	1.3%	1.1%	0.5%	0.3%
S. Hadar	0.4%	0.2%	0.0%	0.3%	0.0%
S. Infantis	0.5%	0.4%	0.3%	0.6%	0.1%
S. Virchow	0.4%	0.5%	0.1%	1.2%	0.0%
S. Agona	0.0%	0.0%	0.0%	1.4%	0.0%
Other serovars	2.9%	3.2%	1.5%	1.8%	0.7%

Table 3 shows that also in relation to broilers/broiler meat, and to layers/eggs, *S.* Enteritidis and to a lesser extent *S.* Typhimurium are associated with the majority of human cases, and the other three regulated types for breeding hens constitute only a small fraction of human cases. Note that in Denmark, the contribution of the non-regulated serovar *S.* Agona via imported chicken was slightly greater (1.4%) than of any of the regulated types except *S.* Enteritidis.

Of the verified foodborne outbreaks of human salmonellosis reported in the EU in 2007 in the context of Directive 2003/99/EC (see Appendix A for further details), 175 (43.4%) outbreaks were attributed to eggs and egg products, including raw eggs in bakery products. The distribution of the implicated serovars is presented in Figure 3.

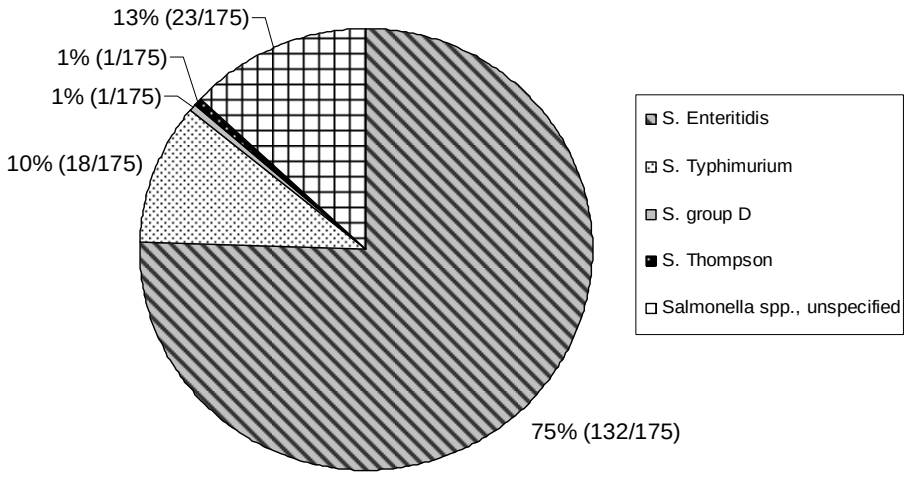


Figure 3. Serovar distribution of *Salmonella* isolates from verified foodborne outbreaks in 2007 linked to consumption of egg and egg products, including raw eggs in bakery products (raw data from EFSA CSR, 2009).

The review of published scientific literature would be another exercise that could help to identify *Salmonella* serovars involved in human salmonellosis outbreaks. A review of peer-reviewed scientific literature published in the period 1970 to 2008 reporting on *Salmonella* serovars involved in foodborne outbreaks linked to the consumption of eggs or egg products is included in Appendix B. Results are summarised in Table 4.

Table 4. Number of human salmonellosis outbreaks reported in literature in humans related to egg or egg products consumption published in the period 1970-2008, with indication of *Salmonella* serovars implicated (Full details in Appendix B).

	***Salmonella* serovar implicated**		
Country	***S.* Enteritidis**	***S.* Typhimurium**	**Other**
EU-27	568	14	2
USA	1116	6	29
Other countries	15	8	1

However, caution has to be taken when interpreting the results:

- These data do not reflect actual number of salmonellosis outbreaks in the countries presented, but the available published literature in peer-reviewed scientific journals on salmonellosis outbreaks identified employing the methodology described in Appendix B.
- The features of the epidemiological investigation methodologies employed for the verification of the source attribution varies between studies, as does the degree of certainty and comparability of the results.

- Some outbreaks may be described in two different publications, as some of this covered a range of time periods in the form of a review.

Nevertheless, these data can be used as further support when identifying those *Salmonella* serovars involved in human salmonellosis outbreaks where egg or egg products consumption was implicated.

Of the verified foodborne outbreaks of human salmonellosis reported in the EU in 2007 in the context of Directive 2003/99/EC (see Appendix A for further details), 15 (3.7%) outbreaks were implicated to 'broiler meat and products thereof'. The distribution of the implicated serovars is presented in Figure 4.

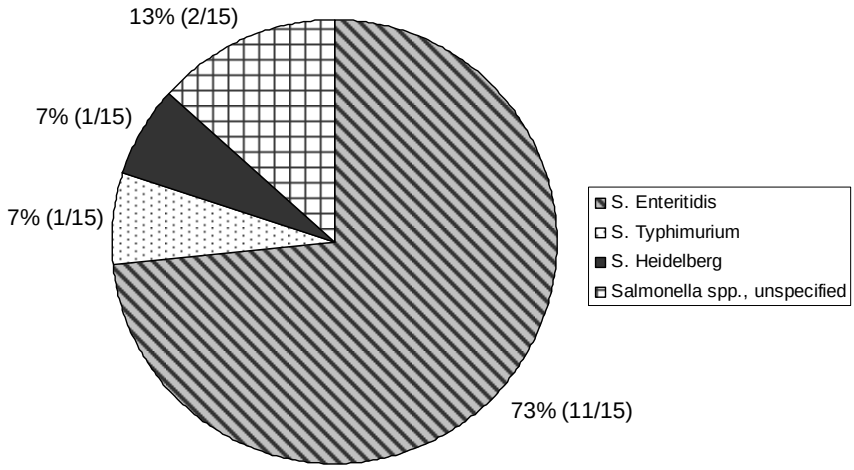


Figure 4. Serovar distribution of *Salmonella* isolates from verified foodborne outbreaks in 2007 linked to consumption of poultry meat and products thereof (raw data from EFSA CSR, 2009).

Following a similar exercise for egg and egg products, a review of peer-reviewed scientific literature published in the period 1970 to 2008 reporting on *Salmonella* serovars involved in foodborne outbreaks linked to the consumption of broiler meat and products thereof is included in Appendix C. Results are summarised in Table 5.

Table 5. Number of human salmonellosis outbreaks reported in literature in humans related to broiler meat and products thereof consumption, published in the period 1966-2008, with indication of *Salmonella* serovars implicated (Full details in Appendix C).

	***Salmonella* serovars implicated**		
Country	***S.* Enteritidis**	***S.* Typhimurium**	**Other**
EU-27	74	1	5
USA	2	3	19
Other countries	1	2	3

The same cautious approach when interpreting this data has to be taken as per the literature review of human salmonellosis outbreaks related to egg and egg products.

2.2.2. *Salmonella* serovars in poultry (*Gallus gallus)* populations in the EU

Data on the prevalence of *Salmonella* in different poultry populations (*i.e.* parent breeding flocks, laying flocks and broiler flocks) and the relative significance of the different serovars isolated are presented in the annex to this opinion (Annex on the analysis of *Salmonella* monitoring and prevalence figures in poultry (*Gallus gallus*) in the European Union between 2004-2007).

Discussion and conclusions on the findings of that analysis are presented in section 4.

2.2.3. *Salmonella* serovars in broiler meat and eggs.

2.2.3.1. Broiler meat and products thereof.

In 2007 a number of MSs have applied monitoring schemes for *Salmonella* in broiler meat on a voluntary basis (The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2007, EFSA, 2009a). A total of 21 MSs and one non-MS[8] reported investigations covering approximately 58,500 units of broiler meat and products thereof, and for 44,000 tested units the sampling stage was specified. The type of products sampled as a unit varied and the analyses were either performed on single samples or on a batch of broiler meats. Data from single samples or from batches exist at all levels of the production chain: slaughter level, processing/cutting level, retail level: depending whether it is fresh broiler meat, or ready to eat (RTE) or non-RTE broiler meat preparations and or products

Overall, 5.5% of the tested units of fresh broiler meat at different levels of the production chain were positive for *Salmonella* in the EU, a decrease compared to the proportion reported in 2006 (6.3%). It has to be noted that these figures are not directly comparable, e.g. due to the variation in the reporting MSs and in the food categories covered over the years. Nevertheless, these reductions do show that progress is already being made (Fels-Klerk *et al.,* 2008; Poirier *et al.,* 2008) and it is likely that targets and control actions set for specific serovars will also reduce the risk of acquisition of other serovars.

In 2007, eleven MSs[9] reported, on a voluntary basis, specific data on *Salmonella* serovar distribution in broiler meat. Overall, *S*. Kentucky was the most frequent serovar reported from broiler meat in 2007 (Table 6). However, this was due to a high number of isolates from Ireland where this serovar was more frequently isolated. It is also dominant in broiler flocks in some companies in Ireland, whereas in another company, *S*. Mbandaka dominates. In this latter company, the serovars were found infrequently on broiler carcasses (Gutierrez et al., 2009). There is also identified a regional cluster of *S.* Paratyphi-B var. Java in the Netherlands, Germany and Luxembourg. This serovar colonised broiler flocks in the late nineteen-nineties, apparently replacing other clones (Van Pelt *et al.*, 2001; Miko *et al.*, 2002). As in previous years, *S*. Enteritidis, *S*. Infantis, *S*. Typhimurium were among the most common serovars in other countries, and the serovar distribution in broiler meat in 2007 was largely comparable to the distribution in 2004 to 2006.

[8] All EU MS except Bulgaria, Cyprus, France, Lithuania, Malta and United Kingdom, plus Switzerland.
[9] Austria, Czech Republic, Germany, Ireland, Italy, Latvia, Luxembourg, Netherlands, Poland, Romania and Slovakia.

Table 6. Distribution of the 10 most frequent *Salmonella* serovars in broiler meat in 2007, as reported by 11 EU MS (EFSA, 2009)

		% positive per country										
Countries	**No. of isolates serotyped**	**S. Kentucky**	**S. Enteritidis**	**S. Paratyphi B var. Java**	**S. Infantis**	**S. Typhimurium**	**S. Hadar**	**S. Virchow**	**S. Agona**	**S. Ohio**	**S. Indiana**	**Other serovars**
Total no. of isolates	**1,494**	**262**	**247**	**153**	**105**	**107**	**70**	**69**	**49**	**29**	**27**	**376**
Austria	96	1.0	35.4	-	21.9	1.0	3.1	-	-	-	4.2	33.3
Czech Republic	53	3.8	34.0	-	-	3.8	1.9	-	9.4	15.1	3.8	28.3
Germany	266	-	26.3	25.2	8.6	7.9	2.3	1.5	-	7.1	5.3	15.8
Ireland	331	77.9	4.2	-	0.9	0.6	-	0.6	10.3	-	0.3	5.1
Italy	201	-	10.0	-	1.5	9.5	14.9	-	-	-	-	64.2
Latvia	21	-	95.2	-	-	-	-	-	-	-	-	4.8
Luxembourg	21	-	19.0	14.3	4.8	33.3	4.8	-	-	-	-	23.8
Netherlands	134	-	3.0	61.9	9.7	1.5	-	4.5	0.7	1.5	4.5	12.7
Poland	283	-	13.8	-	13.8	18.0	5.3	8.5	2.5	-	-	38.2
Romania	75	-	21.3	-	2.7	-	18.7	44.0	-	-	-	13.3
Slovakia	13	7.7	61.5	-	-	15.4	-	-	15.4	-	-	-
Proportion of serotyped isolates		**17.5**	**16.5**	**10.2**	**7.0**	**7.2**	**4.7**	**4.6**	**3.3**	**1.9**	**1.8**	**25.2**

Note: Data are only presented for sample size ≥10. The serovar distribution (% isolates) was based on the number of serovared isolates, including not-typeable isolates and unspecified isolates. Ranking was based on the sum of all reported serovars. Some countries may not have a strict separation of serovars achieved from meat and farm level.

Even though data are incomplete due to the current voluntary reporting system, it can be concluded that the serovar distribution of *Salmonella* on broiler meat is highly diverse, between MS and even within individual MS. The serovars on broiler meat appear to be similar to those found in broiler flocks.

2.2.3.2. Table eggs and egg products.

Several MSs reported data from investigations of table eggs on a voluntary basis (The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union in 2007, EFSA 2009a). In total, 0.8% of the tested units[10] were positive for *Salmonella*, which corresponds to the level found in 2006. Germany and Romania reported most of the investigations of single samples and found 0.7%, 0% of the samples positive at retail, respectively. These MSs reported the majority of data from eggs in 2006 as well.

[10] The definition of unit varies between MS, and usually comprises a number of individual eggs. This number also varies between MS.

Twelve MSs reported results of investigations from retail of table eggs, and on average 0.2% of the approximately 8,500 tested units were positive. The results ranged from 0 to 2.2% in single samples.

Only five MSs reported the *Salmonella* serovar of ten or more isolates from eggs and egg products (based on data from the prevalence tables). *S*. Enteritidis was by far the most dominant serovar reported (54.4%). Several of the other serovars listed among the ten most common have also been reported in low numbers in previous years. However, monitoring was not compulsory in 2007 by Directive 2003/99/EC and three MSs had not applied an official sampling strategy. Furthermore, the type of samples, the frequency of sampling, and the diagnostic methods used throughout the production were not harmonised between the MSs. Egg products may contain materials other than eggs, and may also be subject to cross-contamination.

Due to the scarcity of the data and to the non-harmonised monitoring regimes employed to harvest it, no further conclusions can be made.

2.2.4. Changes in the ability of different *Salmonella* serovars to spread and cause disease in human and animals.

As discussed in section 2.2.1, some changes in the ranking of the frequencies of *Salmonella* serovars involved in human disease have been occurring in the EU as a whole since Regulation (EC) 1003/2003 came into force (i.e. *S*. Hadar has not been among the top 5 in the last two reported years), the two most frequent serovars are still remaining *S*. Enteritidis and *S.* Typhimurium from salmonellosis cases in humans (see previous Table 2).

Beside these general observations there are tendencies in the frequencies of isolated *Salmonella* serovars in some countries, which should not escape our attention, even though they are not influencing the overall picture at the moment. One example is the emergence and dominance of multidrug-resistant clone of *S*. Infantis in broilers produced in Hungary, which has been accompanied by an increased prevalence of this serovar in the human population (Nógrády *et al.*, 2007). An increased prevalence of *S*. Infantis has also been reported in broilers by Austria and Poland (Table 6).

Another example is the increasing prominence of the multidrug-resistant *S.* Paratyphi B variant Java in human outbreaks in several European countries (Denny *et al.*, 2007). This serovar has been isolated with high prevalence from poultry and poultry products in Germany and the Netherlands (see Table 6, and Dorn *et al*., 2001, Miko *et al*., 2002). Recent data from the Ireland are reporting the dominance of *S*. Mbandaka and *S.* Kentucky in broiler flocks, although an epidemiological link between the animal and human strains could not be established so far (Gutierrez *et al*., 2008).

Such changes in dynamics and patterns of the spread and in the role in outbreaks *Salmonella* serovars have been occurring and will probably continue to occur in the future. Although the role of increased pathogenicity of *S*. Typhimurium DT104 for different animal species is still not clear, resistance to therapies due to frequent multidrug-resistance of this serovar is considered an important advantage of this pathogen for survival and spread. For *S.* Enteritidis and in particular phage types PT4 and PT8 in poultry, there are several epidemiologic and clinical data from the past indicating an increased virulence and/or increased ability to colonize the oviduct of breeders and layers, and to spread (Poppe, 2000). It is this ability of *Salmonella* serovars for vertical transmission that will determine their importance in breeding flocks.

2.2.5. Increased virulence or resistance to relevant therapies for human salmonellosis

2.2.5.1. Virulence of different *Salmonella* serovars.

In general, the course of non-typhoid salmonellosis in humans is characterized by a clinical picture which may include fever, diarrhoea, abdominal pain, nausea and vomiting. Hospital admission may sometimes be required. In infants and young children, in the elderly and immunologically suppressed, some fatal cases may occur. However, mortality rates still largely unknown and data are not readily available (ECDC, 2008).

One recently published study reports that *Salmonella* serovars that are closely related genetically may differ significantly in their pathogenic potential (Jones *et al.*, 2008). Definitive evidence for increased virulence of specific serovars or of subtypes or clones for humans is thus very difficult to obtain. However, a significant excess mortality up to one year after infection with zoonotic *Salmonella* such as *S*. Enteritidis, *S*. Typhimurium and *S*. Dublin has been observed in Denmark in the past (Helms *et al*., 2003). Some studies have shown that the clinical symptoms of multi-drug resistant *S*. Typhimurium DT104 are more severe than other *S*. Typhimurium or *S*. Enteritidis infections, and due to their antimicrobial resistance, are difficult to treat (Helms *et al*., 2002). There are data indicating an increased virulence of some further invasive non-typhoid *Salmonella* serovars such as *S*. Dublin, *S*. Virchow, *S*. Heidelberg, and *S*. Choleraesuis (Wollin *et al.*, 2007).

2.2.5.2. Increased antimicrobial resistance

Antimicrobial resistance of non-typhoidal *Salmonella* has been increasing over the last two decades, although the level and extent of resistance vary according to different regions and to different serovars. Several mobile genetic elements (i.e. plasmids, transposons, genomic islands) play an important role in the horizontal transfer of antimicrobial resistance, thereby helping resistance determinants to spread among bacteria due to horizontal gene transfer.

International organizations beside EFSA (*i.e.* WHO, OIE, EMEA) survey the problem from time to time, and provide analysis of the data. Recently the *Codex Alimentarius* Commission has established an Ad Hoc Intergovernmental Task Force on Antimicrobial Resistance which had two sessions in Seoul, Korea (2007, 2008). The aim of the Task Force is to develop science based guidance to assess the risk of human health associated with the presence of antimicrobial resistant microorganisms and resistance determinants. According to the Directive 2003/99/EC resistance to antimicrobial agents should be monitored in zoonotic bacteria including *Salmonella* and in commensal (indicator) bacteria. EU member states presently generate and report these data in different ways. Examples of integrated reporting systems include DANMAP in Denmark[11] and NETHMAP in the Netherlands[12].

Most often the reported *Salmonella* isolates constitute a sub-sample of isolates available at the National Reference Laboratory. Isolates may be obtained by different monitoring approaches, either by active and systematic monitoring of humans, animals, foods, and other sources, or by passive monitoring based on diagnostic submissions of samples from clinical cases in animals and by testing of foods on suspicion. Some countries like the UK and the Netherlands do report with some further detail data on antimicrobial resistance for some *Salmonella* isolates (representing limited serovars) from human salmonellosis, while in the Danish report there is

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

no information down to *S*. serovar level except for *S*. Enteritidis and *S*. Typhimurium ((Hammerum *et al*., 2007; HPA, 2006 and 2008; NETHMAP, 2006, 2007 and 2008).

The serovars from isolates in the UK that are most frequently showing multidrug-resistance (MDR) are *S*. Typhimurium, *S*. Paratyphi-B (d-tartrate-positive *Salmonella enterica* serovar Paratyphi B), *S*. Hadar, *S*. Virchow, *S*. Heidelberg, *S*. Newport and certain clones of *S*. Infantis (McDermott, 2006). MDR seems to be most characteristic to *S*. Typhimurium (Table AB-SA2, The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union in 2007, EFSA, EFSA 2009a). The MDR region of *S*. Typhimurium DT104 resides in the 46 kb genomic island (SGI-1), which has been shown to be transmissible by P22-like phages (Cloeckaert, and Schwarz, 2001). Such strains are characterized by the pentaresistant (ACSSuT) phenotype (McDermott, 2006).

In contrast to *S*. Typhimurium, MDR in *S*. Enteritidis is relatively rare as evidenced by retrospective studies in Italy and in the UK, and in almost all member states (Table AB-SA1, The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union in 2007, EFSA, EFSA 2009a). However, there is an increasing trend for appearance of nalidixic acid and fluoroquinolone resistance among both *S*. Typhimurium and *S*. Enteritidis of human and poultry isolates (Fisher, 2004), which can also be observed between similar data about human isolates of these two serovars from the year of 2005 to 2006 (EFSA, 2006; EFSA, 2007). This is especially true for *S*. Enteritidis and *S*. Hadar in which serovars the acquired resistance to quinolones has also been related to plasmid mediated Qnr type (Cattoir *et al*., 2007).

Increasingly, microbiologists and clinicians are faced with bacteria that produce enzymes able to hydrolyse third generation cephalosporins, such as cefotaxime and ceftazidime, which are used widely in empirical and specific regimens for human bacterial sepsis. These so-called extended-spectrum b-lactamases (ESBLs) and AmpC b-lactamases are usually encoded by genes present on transferable plasmids, which often encode resistance to other antibiotic classes, such as aminoglycosides, trimethoprim. An example of a recent "epidemic" is the case of *Salmonella* Newport in the USA and Canada (Gupta *et al.*, 2003; Weir, 2004).

Currently, EFSA has outsourced the analysis of the information collected from the European Union MSs and certain other European countries on the occurrence of antimicrobial resistance. In particular, for *Campylobacter, E. coli* and enterococci as well as *Salmonella* serovars and phage types isolated from animals or foodstuffs and reported under the Council Directive 2003/99/EC or in the context of the EU-wide baseline surveys. A report on temporal and/or spatial trends on antimicrobial resistance is planned to be available by late 2009.

2.2.5.3. Antimicrobial resistance in isolates from poultry *Gallus gallus.*

The occurrence of antimicrobial resistance in *Salmonella* in poultry production in the EU and foodborne antimicrobial resistance as a biological hazard were reviewed in previous EFSA Opinions (EFSA, 2004a; EFSA, 2008b).

Recently published data indicate that the occurrence of resistance in *S.* Typhimurium and *S.* Enteritidis isolates from humans resembles the occurrence of antimicrobial resistance reported for these serovars in poultry (*Gallus gallus*) (Tables AB-SA5 and AB-SA6, The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents, in the European Union in 2007, EFSA 2009b). Also here, it is remarkable that MDR is more than 10 times more frequent in *S.* Typhimurium as compared to *S.* Enteritidis, while quinolone (nalidixic acid) resistance is about twice as frequent in *S.* Enteritidis as in *S.* Typhimurium.

Due to the low prevalence, a very scant amount of data concerning the antimicrobial resistance of the less common serovars was expected. However, even considering the most common serovars, namely *S.* Enteritidis and *S.* Typhimurium, it is difficult to make any inference because of the lack of data homogeneity and data stratification among the monitoring programmes of the different MS. Even the situation of antimicrobial resistance according to the category of animals (layers or broilers) is a level of detail that the MSs seldom report.

Therefore EFSA (EFSA, 2007) proposed a harmonized monitoring scheme of antimicrobial resistance in *Salmonella* in *Gallus gallus*. Also, it proposed a common set of antimicrobials to test using common epidemiological cut-off values to determine the susceptibility of *Salmonella* and *Campylobacter*. Based on the Decision of Commission (2007/407/EC), results of the harmonized monitoring should be reported in accordance with Article 9 of Directive 2003/99/EC, in the yearly report on trends and sources of zoonoses, zoonotic agents and antimicrobial resistance.

This is especially important because the two most frequently isolated *Salmonella* serovars from human salmonellosis cases (*S.* Typhimurium and *S.* Enteritidis) are showing an increasing frequency of their antimicrobial resistance determinants, especially fluoroquinolones. Besides – as mentioned above – there are other serovars with MDR, and these are found in unusual high prevalence in the poultry industry of certain member states. These aspects indicate that, at least in some MSs, such serovars should be considered as of special concern for human health.

At the same time, recent reviews present data on *Salmonella* isolates from poultry and poultry products carrying b-lactam resistance genes (Batchelor *et al.*, 2005; Li *et al*., 2007; Torres and Zarazaga, 2007; Cloeckaert *et al.*, 2007).

3. Literature review on transmission of *Salmonella* in the poultry production chain

3.1. Description of the poultry production chain

The poultry industrial poultry production structure has been described in a previous EFSA Scientific Opinion (EFSA, 2004b). There are two main food production systems: poultry meat (carcasses and processed products), and eggs for consumption (table eggs) and further processing (egg products).

Various species are used in industrial poultry meat production: chickens (broilers) (*Gallus gallus*), turkeys, ducks and guineafowl, their importance varying with regions and food customs. Some alternative production systems also exist, such as organic and free-range production.

In 2007, 11.5 million tons (Tons Equivalent Carcasses) of poultry meat were produced in the EU, mainly broilers (75%), turkeys (16%) and ducks (4.4%). This EU poultry meat production represents the second important meat species production and EU is actually the third producer in the world, producing 13 % of the world poultry meat (AVEC, 2008). Nevertheless the importation of poultry meat from third countries is increasing (+29% in 2007).

For the egg line the production evaluated, in the EU is approximately 6.61 million tons, representing 101,400 million eggs produced per year for the human consumption.

Production of poultry meat or eggs (Figure 5) is based on selection of male and female pure lineages on very precise genetic criteria, such as productivity, quality of products and resistance against disease. The selection methods assure a uniform quality of bird for further multiplication and production. Selection criteria differ according to the types of production. After the incubation time of eggs stemming from this first crossing, the chicks are raised in breeding steps, giving rise to chicks intended for fattening for poultry carcasses, and pullets for laying of eggs for human consumption. The selected offspring from these are then multiplied in great-grandparent flocks and grandparent flocks which are maintained at high biosecurity. Chicks from grandparent flocks are used to populate parent flocks, *e.g.* broiler or layer breeder flocks, which are normally held by individual commercial companies. Eggs from these parent flocks are then hatched in commercial hatcheries to produce the commercial generation of birds.

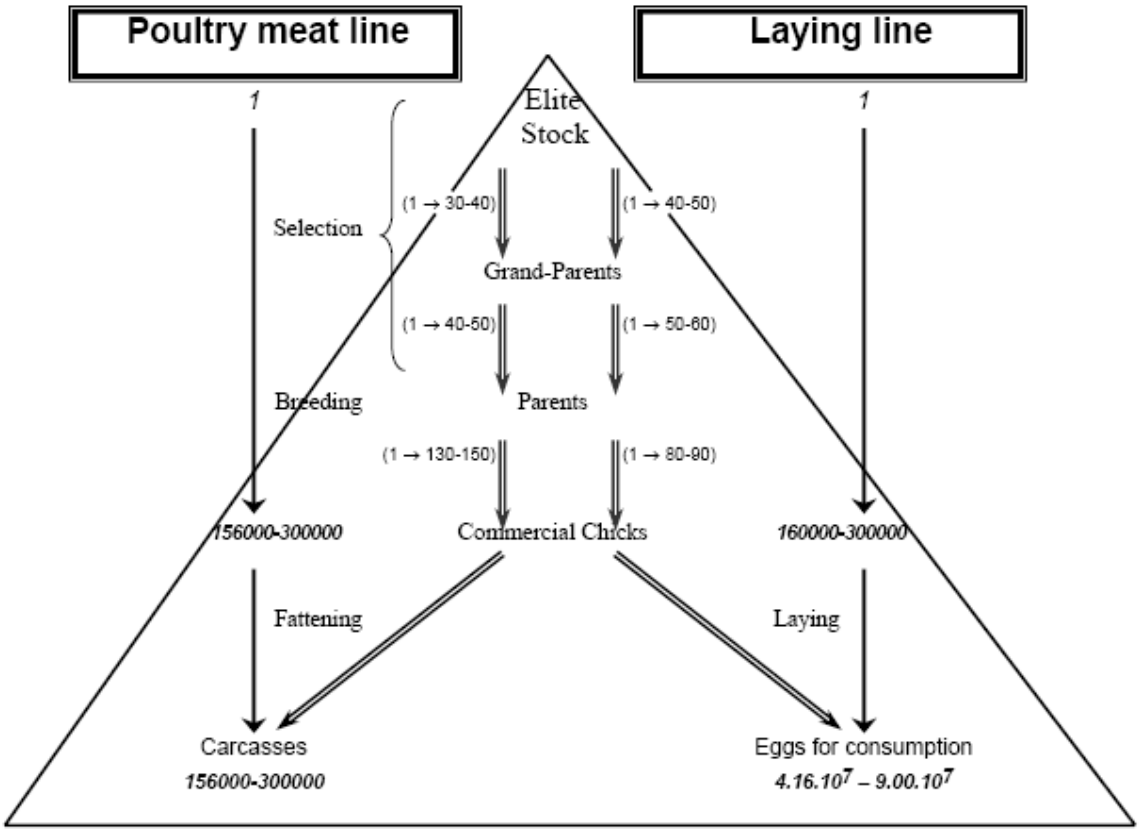


Figure 5. Simplified structure of poultry production. For explanation see text below (Modified from EFSA, 2004b)

Different genetic lines of birds are used for meat and egg producing flocks of chickens. Moreover, genetically male and female lines may be more specialised so as to contribute carcase characteristics and fecundity, respectively. There are also different genetic lines of birds for conventional and free-range or organic production systems.

The structure is "pyramidal". Every stage engenders a consequent reproduction of the number of individuals of the following stage: for example, at the selection step (Elite stock and grand parents), every hen produces 30 to 60 chicks. Afterward, at the stage of breeding, this multiplication factor is increased and can reach 80-90 laying hens or 130 to 140 broilers. Because of this mode of production, theoretically every Elite female could be the origin of

between 156,000 and 280,000 broilers or between 160,000 and 300,000 laying hens producing between $4.16x10^7$ and $9.00x10^7$ table eggs.

Another estimate (Hunton, 1993), calculates that the multiplication of birds and products from a single breeding bird results in approximately 180,000 table eggs or 250 kg of meat being derived from each female bird in parent flocks for layers and broilers respectively.

Intense genetic selection is carried out in primary breeding or elite flocks to achieve ongoing progress in terms of performance characteristics. These flocks are normally kept under conditions of extremely high biosecurity and in the case of chickens, normally in regions where there is a low prevalence of *Salmonella* spp. and a low risk of other notifiable avian diseases that may threaten the health status of the flock.

Due to the economical high value of breeding poultry, breeding steps (selection and breeding) are managed by very few companies, all over the world. Consequently there is a very large exchange of materials (fertilised eggs, one day old chicks) not only at the E.U. level, and grand-parents and parents are distributed worldwide to be hatched and reared in another place. Moreover, there is an active intra and extra community movement of utility poultry lines (*i.e.* laying hens and broilers).

Data on the export and import of poultry (intra and extra community trade) are compiled and available in a particular EUROSTAT database[13]. Information on these activities is reported by the different MSs at their discretion on voluntary bases, and thus caution should be exercised as underreporting would probably be quite common.

Overall and according to EUROSTAT data, the intra-community trade of both parent lines and production lines is considerably higher than extra-community imports of birds. At the same time, there is a considerable variability between MSs. For example, countries like France, Spain, The Netherlands, Greece and the United Kingdom report a considerable number of movements of both breeding and production chicks (up to the magnitude of 10^6 for same types), while the other EU MSs barely report any movements. There are control measures in place specified in regulatory instruments for the trade in poultry and hatching eggs (Council Directive 90/539/EEC of 15 October 1990 on animal health conditions governing intra-Community trade in, and imports from third countries of, poultry and hatching eggs; Commission Regulation (EC) No 798/2008 of 8 August 2008 laying down a list of third countries, territories, zones or compartments from which poultry and poultry products may be imported into and transit through the Community and the veterinary certification requirements).

3.2. Epidemiology of *Salmonella* in poultry production

Numerous factors (*e.g.* poultry, humans, rodents, wild birds, insects, water, feed) can introduce infection into a poultry unit and salmonellas can spread from unit to unit through movements of vehicles, equipment and utensils, including egg trays contaminated with the organism (Figure 6).

There are numerous routes by which *Salmonella* can contaminate chicken products (meat and eggs) by the time they enter the human food processing and retail chain. Considering the stages of broiler production in reverse order, carcass contamination may be acquired via one

[13] EUROSTAT dedicated database available at: http://epp.eurostat.ec.europa.eu/portal/page?_pageid=1073,46870091&_dad=portal&_schema=PORTAL&p_product_code=APRO_EC_POULA

or more of: endemic strains in slaughterhouse and carcass processing machinery (Chriél *et al.*, 1999; Heyndrickx *et al.*, 2002), inadequately decontaminated transport crates, (Chriél *et al.*, 1999; Heyndrickx *et al.*, 2002; Heyndrickx *et al.*, 2007) or *Salmonella* already present in the production flock (Bailey *et al.*, 2002; Corry *et al.*, 2002; Liljebjelke *et al.*, 2005). Flock infections can result from carry-over in the farm environment from previous flocks (Davies *et al.*, 2001), carry-over or introduction via wildlife (Davies and Wray, 1995; Henzler and Opitz, 1992) or feed (Chriél *et al.*, 1999; Corry *et al.*, 2002; Davies *et al.*, 2001), and some *Salmonella* will arrive with infected chicks from the hatchery.

Infected broiler and laying hen chicks may acquire *Salmonella* infections via 'vertical' transmission in the egg from the parent breeder flock, or via 'horizontal' transmission from contamination in the hatchery. Vertical and horizontal routes are interlinked by interactions, as discussed further down.

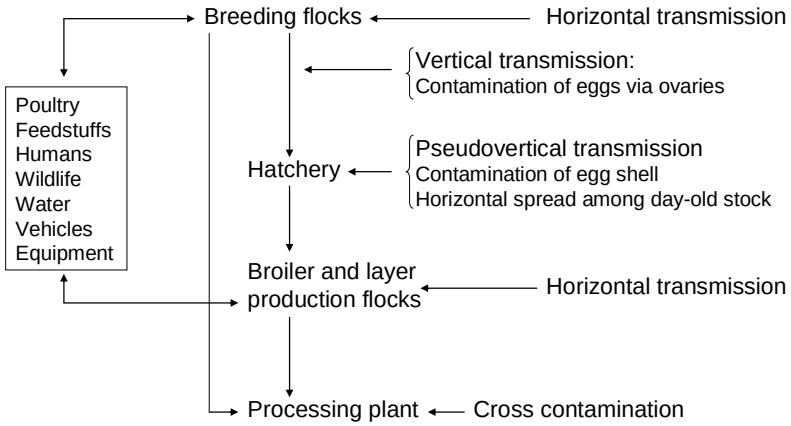


Figure 6. Potential routes of *Salmonella* infection and contamination (Modified from Poultry Diseases, F.T.W. Jordan, 3rd Edition, 1990)

The naming of the different pathways by which eggs (in general) can become contaminated with bacteria (including *Salmonella*) varies through the literature. In a EFSA opinion published in 2005 on Microbiological risks on washing of table eggs (EFSA, 2005b), bacterial contamination of shell eggs was defined as occurring either vertically (direct deposition during egg formation when still attached to the ovary) or horizontally (divided in either internal – before shell formation – and external – after shell formation).

In a recent EFSA opinion on cooling of eggs (EFSA, 2009b), the contamination of table eggs with *Salmonella* was categorised as primary contamination (before shell formation) and secondary contamination (contamination of the shell surface and following penetration of bacteria from the shell into the egg content).

In the context of this opinion, the following meaning will be used for the different pathways by which hatching eggs can become contaminated:

- *True vertical transmission*. From parents to progeny via internally contaminated eggs, due to colonisation of the reproductive organs (ovary or oviduct) or due to penetration into the forming egg, within the body of the hen as it passes down the oviduct and is voided from the cloaca. This is most likely to occur with *S.* Enteritidis (or the non-zoonotic serovar *S.* Gallinarum).
- *Pseudo-vertical transmission*. From parents to progeny via externally contaminated eggs. Egg shells can be contaminated by faecal material on egg belts, in nest boxes or by handling equipment or personnel. It is possible for *Salmonella* to be drawn inside intact or cracked eggs by the pressure gradient that forms during cooling from body temperature, but a high level of contamination and available water is necessary for this. Such contamination can multiply during incubation and be released from egg- shell membranes during hatching, resulting in hatchery-acquired infection in chicks.
- *Horizontal Transmission.* From other animals in the same layer of the production pyramid, or from other sources, such as contaminated feed. Spread of *Salmonella* from a previously contaminated hatchery, poorly disinfected transportation or accommodation or persistent infection in wildlife vectors such as rodents or litter beetles, personnel or equipment can all be involved in horizontal spread, although some infections can occur at very low within-flock prevalence and resolve spontaneously within a short period.

Once a breeding flock is infected with *Salmonella* it can be very difficult to eliminate the bacteria from the production and they can spread to other units via hatcheries by both vertical and horizontal spread. Strategies for the control of *Salmonella* in poultry in primary production have been previously reviewed in the EFSA Opinion on the use of vaccines for the control of *Salmonella* in poultry (EFSA, 2004b).

Carrier states of *S.* Enteritidis and *S.* Typhimurium have been described in chickens (Barrow *et al.,* 1987; Sadeyen *et al.,* 2004). The hosts show no clinical symptoms and the bacteria are shed intermittently. Chickens infected but shedding bacteria at a very low rate or intermittently might be undetectable through sampling as requested through the surveillance programmes. Epidemiologically these animals are of great importance since they are not recognized as infected but are able to transmit the infection to susceptible individuals. Transmission of *Salmonella* from parent stocks to broiler flocks may occur either by true vertical transmission, by faecal contamination of the eggs (pseudo-vertical transmission), or through horizontal transmission in the hatchery (Gast, 2007).

3.2.1. True vertical transmission of *Salmonella* to hatching eggs

Hatching eggs from flocks infected with *Salmonella* are usually sterile in their internal contents when laid, but occasionally, infected breeding flocks can pass the organism via the egg to the progeny. *S.* Enteritidis has a high ability to colonize the ovary and the preovulatory follicles causing transovarian transmission of *S.* Enteritidis (Gantois *et al.,* 2008a) and has even been isolated from the reproductive organs of infected hens in the absence of caecal colonization.

Information on the mechanisms of vertical transmission is incomplete, and there appears to be more than one way in which an egg may be internally contaminated by the time it arrives at a hatchery. Systemic infections of breeding hens with *Salmonella* can certainly result in the infection of the reproductive tract, both in experimental (Cox *et al.*, 2000) and field (Barnhart *et al.*, 1991; Corkish *et al.*, 1994; Hoop and Pospischil, 1993; Lister, 1988) studies.

On the other hand, experimental inoculations in laying hens by a variety of routes, including intravenous, conjunctival, cloacal and vaginal, have resulted in detectable *Salmonella* in the ovaries and the oviduct, providing routes for *in vivo* contamination of yolk and albumen, respectively (Cox *et al.*, 1973; Cox *et al.*, 2000; Gantois *et al.*, 2008; Miyamoto *et al.*, 1997; Okamura *et al.*, 2001a; Okamura *et al.*, 2001b). However, relatively few *Salmonella* serovars appear to be able to consistently infect eggs laid by these experimentally inoculated hens, suggesting that there are other critical factors determining an invasive *Salmonella* strain's potential for internal egg contamination (Berchieri *et al.*, 2001; Keller *et al.*, 1997). Some reports have suggested that survival within the albumen of the forming egg is such a factor, as certain commonly egg-transmitted serovars, such as *S*. Enteritidis (SE), are consistently well-adapted in this respect (Gantois *et al.*, 2008; Gast *et al.*, 2004; Gast *et al.*, 2007).

3.2.2. Pseudo-vertical transmission of *Salmonella* to hatching eggs

Contamination is much more likely to happen through faecal contamination of the surface of the egg. As the egg passes through the cloaca, *Salmonella* in faeces attach themselves to the warm surface of the shell and are drawn inside as it cools if excess moisture is present. Fertile inoculated eggs hatch despite high levels of *Salmonella* and may infect other chicks in the same hatcher cabinet or airspace (hatcher room, chick holding area) and can reach the gut of other chicks hatching from *Salmonella* free eggs before they are removed from the hatcher.

Following the infection of a hen with *Salmonella*, the laying of internally infected eggs may happen for a relatively short period of time (Berchieri *et al.*, 2001), and some investigators consider that much internal contamination of eggs may be acquired across the shell after lay (Cox *et al.*, 2000; Messens *et al.*, 2005). The penetration of freshly laid eggs by *S.* Typhimurium, with contamination to the point of hatching, was very successful regardless of whether it was applied by spray or by contact with dry, contaminated litter (Padron, 1990). This suggests that systemic infection of breeding hens is not a necessary precondition for eggs and hatching chicks to be internally infected by *Salmonella*, provided that the organism comes into contact with the surface of freshly laid eggs, whether via faecal, vaginal or environmental contamination, and the strain is one that can survive once it has penetrated the eggshell. Under those circumstances, this 'pseudo-vertical' transmission has a similar outcome to true vertical transmission via ovaries or oviduct.

3.2.3. Horizontal transmission of *Salmonella* to hatching eggs

Salmonella is able to persist in the environment and is capable of surviving more than a year in empty, cleaned and disinfected poultry houses, and more than two years in poultry feed. *Salmonella* can survive in the surroundings of the houses in small pockets of litter and fan dust making reintroduction possible. Persistent *Salmonella* in the house and its surroundings have been shown to be significant factors in relation to infection of parent stocks, layer and broiler flocks, and lack of cleaning and disinfection of areas surrounding the entrance of the houses increase the risk of *Salmonella*.

Given that systemic hen infection and environmental contamination are aspects of the same problem, and that either may lead directly to internally-contaminated eggs leaving a breeding farm, investigations have examined the environmental distribution of *Salmonella* on affected breeding units. Areas commonly contaminated have included feed hoppers and pipes, and ventilation systems (Davies and Wray, 1996; Davies *et al.*, 1997; Davies *et al.*, 1998). Of potentially high significance, in view of egg contact, is the tendency for contamination of nest

boxes and of equipment and areas dedicated to egg handling (Davies and Wray, 1996; Davies *et al.*, 1998; Kim *et al.*, 2007). Cleaning and disinfection will often fail to eradicate contamination (Kim *et al.*, 2007), even in areas (such as egg handling and storage) perceived to be 'clean' (Davies and Wray, 1996; Davies *et al.*, 1998; Heyndrickx *et al.*, 2002).

3.3. The relative importance of vertical and horizontal transmission

3.3.1. *Salmonella* in breeding flocks and hatcheries

It is not always possible to distinguish infections originating in the hatchery from those originating in the breeding flock, but traceback exercises (Liljebjelke *et al.*, 2005; McIlroy *et al.*, 1989) and risk factor analyses (Chriél *et al.*, 1999; Skov *et al.*, 1999) show that breeding flocks can be identified as the source in many cases. Amongst commercial layers, contaminated eggs will typically result from flock infections acquired via persistent environmental and wildlife-associated *Salmonella* (van de Giessen *et al.*, 1994; Wales *et al.*, 2006), although feed and replacement pullets are other possible routes.

The occurrence of *Salmonella* high in the poultry production stages (see previous section 3.1. and Figure 5) can lead to widespread infection throughout production (Hensel and Neubauer, 2002). This means that the occurrence of any *Salmonella* at elite or grandparent level is serious and should not be tolerated. In North European countries such flocks are extensively tested and removed from production if infection with any *Salmonella* serovar occurs. *Salmonella* control at the parent breeder and associated hatchery level is less rigorous, and is focused upon certain serovars. Directive 2003/99/EC requires monitoring of poultry breeding flocks and hatcheries for *S.* Enteritidis and *S.* Typhimurium as well as other serovars considered by virtue of their frequency in humans to be of special public health significance. Although chicks originating from domestic elite or grandparent flocks in most EU countries are normally free from *Salmonella* they may become infected during the rearing or laying stage through any of several routes, and particularly via contaminated feed except possibly in the case of *S.* Enteritidis which is rarely identified in feed, but may be underestimated. Currently, the predominant causes of infection of parent flocks with *S.* Enteritidis and *S.* Typhimurium are not known.

The sources of *Salmonella* infection of parent breeder flocks are similar to those of production flocks, although the relative importance of the various sources differs, in accordance with the generally higher hygiene and biosecurity afforded to breeding flocks and within individual companies (Kwag *et al,* 2008). Vertical transmission from infected elite and grandparent breeding flocks is rare (McIlroy *et al.*, 1989; Davies *et al.*, 1997; Davies *et al.*, 2003), although it has been reported (Brown *et al.*, 1992) and was not always the case (Cox et al 1991). Hatcheries can infect breeder flocks, either due to contamination in the hatchery supplying eggs to the breeder rearer (Cox *et al.*, 1991), or by back-transfer on equipment and personnel from hatcheries supplied by the breeder flock (Davies *et al.*, 1997). Inadequate cleaning and disinfection or persistent contamination in the adjacent external environment or ventilation system can, as with production flocks, lead to recurrent infection in breeder flocks (Davies *et al.*, 1997). In view of the high biosecurity status of breeder flocks, feed supplies are generally carefully protected and typically heat-treated. Above parent level additional acid treatment of feed is also often used. Nonetheless, feed- or feed mill-associated flock infections are not uncommon (Davies *et al.*, 1997; Henken *et al.*, 1992). Possible factors in feed-associated infections include smaller feed mills, which may not have dedicated breeder feed production and storage lines (Henken *et al.*, 1992), and the inherent technical difficulties in

hygienically cooling and storing heat-treated breeder meal rations (EFSA, 2008c), compared with the pelleted rations typically used for broilers. In addition, the relatively long life of broiler breeder flocks compared with production flocks gives a longer opportunity for contamination to arrive in feed and establish within a flock, thence to disseminate to the hatchery and beyond.

3.3.2. *Salmonella* serovar differences in their tendency for vertical transmission

Particular serovars are more commonly found to contaminate hatching eggs internally, the typical example of which is *S.* Enteritidis, which is more consistently traced from breeders to broiler carcasses than are other serovars (Byrd *et al.*, 1998; Kim *et al.*, 2007). However, *Salmonella* serovars other than *S.* Enteritidis trace-backs to breeders have been documented, including *S.* Typhimurium, *S.* Heidelberg, *S.* Kentucky and *S.* Senftenberg (Byrd *et al.*, 1998; Chriél *et al.*, 1999; Kim *et al.*, 2007; Liljebjelke *et al.*, 2005). By contrast, hatchery contamination leading to carcass contamination seems commonly to involve a wide range of serovars (Bailey *et al.*, 2001).

S. Enteritidis was compared with five other serovars (*S.* Typhimurium, *S.* Infantis, *S.* Heidelberg, *S.* Hadar and *S.* Montevideo) in an intravenous infection model (Okamura *et al.*, 2001a). *S.* Enteritidis colonised the caecum and internal organs (including the reproductive organs) in higher numbers and for longer than the other serovars, and was the only one found to have infected eggs internally. The same serovars were administered vaginally, and again *S.* Enteritidis was recovered more frequently, and in higher numbers, than the other *Salmonella*e from spleen, ovary, oviduct and egg contents, although on this occasion *S.* Typhimurium also contaminated eggs (Okamura *et al.*, 2001b). Serovars *S.* Typhimurium, *S.* Senftenberg and *S.* Thompson appeared to be poor internal colonisers of eggs in an oral inoculation study (Cox *et al.*, 1973). However, a view that *S.* Enteritidis is simply a better systemic coloniser of hens than other serovars, and consequently tends to be deposited more often in forming eggs, is not supported by certain other evidence and much depends on the characteristics of the individual strains chosen for such experiments and their physiological state after storage. In a survey of 42 spent layer flocks in the USA, ovarian infection by *Salmonella* was quite common, but a wide variety of serovars were found, and *S.* Enteritidis was isolated in only a small minority of these cases. Furthermore, in oral and intravenous inoculation models, *S.* Heidelberg (a problem serovar for egg contamination in the USA) and certain *S.* Typhimurium strains appear to colonise the chicken reproductive organs as well as *S.* Enteritidis in experimental models, despite being recovered less often from egg contents (Gantois *et al.*, 2008; Gast *et al.*, 2004; Gast *et al.*, 2007).

Examination of a *Salmonella* strains survival in albumen at chicken physiological temperature appears to provide some insight into a factor which may determine egg contamination sufficient to support vertical transmission. In one study (Gantois *et al.*, 2008) the chicken egg-associated serovars of *S.* Enteritidis, *S.* Typhimurium and *S.* Heidelberg were clearly superior to the non-egg-associated *S.* Hadar and *S.* Virchow serovars in this respect, whereas measures of systemic colonisation by the various strains were uninformative. The pre-eminence of *S.* Enteritidis in egg contamination may relate to the observation that this highly clonal serovar is consistently good at survival in albumen, whereas *S.* Typhimurium, for example, shows more inter-strain variation (Gantois *et al.*, 2008; Messens *et al.*, 2005). A genetic locus identified in *S.* Enteritidis (*yafD*) appears to confer an enhanced phenotype for survival in albumen, which was transferable to a *S.* Typhimurium strain (Lu *et al.*, 2003). Other factors proposed to be important for intra-egg survival and access to the yolk (permitting multiplication to high

numbers) include high molecular mass lipopolysaccharide and surface appendages such as curli, type I fimbriae and flagella (Cogan *et al.*, 2004; De Buck *et al.*, 2004; Guard-Bouldin *et al.*, 2004; Guard-Petter, 2001).

3.3.3. Hatcheries and horizontal transmission

Hatcheries sanitise, incubate and hatch eggs from breeding farms, and will then sort chicks, destroy males from layer breeders, and dispatch the chicks in boxes or crates. Eggs are handled at several stages including at arrival, sanitising, setting in incubators, candling to check viability, and transfer to hatching incubators. Areas of hatcheries that have been identified as being prone to persistent *Salmonella* contamination include: ventilation ducts of incubators, incubator door seals, egg transfer machines, egg tray and hatcher/delivery basket washers, chick handling areas and waste processing areas (Davies and Wray, 1994; Davies *et al.*, 2003; Davies and Breslin, 2004; Kim *et al.*, 2007). In some cases, strains of *Salmonella* that are found in hatcheries may be traced to the supplying breeder flocks (Corkish *et al.*, 1994; Davies and Breslin, 2004). However, in other studies, endemic hatchery strains may not resemble those currently found 'upstream' in breeders (Bailey *et al.*, 2002), but breeding flocks are likely to have been the original source. The endemic contamination of hatcheries may occur with multiple serovars simultaneously but often there are one or two predominant serovars which may persist for years (Byrd *et al.*, 1999). The situation is quite complex as some serovars found in breeding flocks may never be found in the hatchery (Bailey *et al.*, 2002), others found in the hatchery may have no obvious link with breeding flocks, some serovars may be transient residents of the hatchery and others more permanent and not all serovars found in the hatchery are significant colonisers of the chick output.

Salmonella contamination appears to be especially difficult to control in hatching incubators (hatchers) and waste handling areas (Davies *et al.*, 2001; Davies and Breslin, 2004; Kim *et al.*, 2007). Most contaminated eggs have *Salmonella* on the shell surface only, and therefore eggs are usually sanitised using a variety of methods and agents, including formaldehyde based products, chlorine, quaternary ammonium compounds, hydrogen peroxide and polyhexamethylene biguanide (PHMB) (Berrang *et al.*, 2000). Synthetic phenolic products are also very effective for surface decontamination but fumigation by formaldehyde vapour in transit or on entry to the hatchery is the method of choice. Sanitising methods aim to achieve a high kill of *Salmonella* with penetration of organic matter and shell pores, but efficacy may be constrained by the need to avoid reducing hatchability. Good egg sanitisation can reduce contamination in egg handling areas, but it has a limited effect on contamination in hatchers, which may then circulate between machines via contaminated dust, and aerosols generated during power-washing (Bailey *et al.*, 1996; Davies and Wray, 1994). The ability to strip out and access the interiors of incubators and associated ventilation ducting for C&D is important for preventing and eliminating contamination in these areas (Davies and Wray, 1994). Control of *Salmonella* in hatcheries has become more problematic in recent years since the Health and Safety Policies operated by poultry companies have led to the use of less noxious but less effective disinfectants (Mitchell and Waltman, 2003), such as amphoteric surfactants, peroxygens or quaternary ammonium compounds which may be more easily inactivated by residual organic matter. Failure of implementing adequate GMP allows resident *Salmonella* to become established, particularly in ventilation ducting and beneath door seals of hatcher incubators. The disinfectants may also not be effective at standard concentrations in tray wash machines so there is a danger of contamination of hatcher baskets, delivery baskets and egg trays if this is not properly controlled (Davies *et al.*, 2001).

Probably the most critical area for the horizontal spread of *Salmonella* in a hatchery is the hatching incubator. It is here that both internal and external egg contamination will be disseminated by aerosol and by airborne chick down (fluff) as the chicks hatch, especially if there are several batches of eggs due to hatch on different days within the same hatcher room. Experimentally, *S.* Typhimurium applied externally to a small proportion of hatching eggs before incubation led to heavy contamination of the hatcher air around the time of hatch and widespread infection of newly-hatched chicks (Bailey *et al.*, 1996; Cason *et al.*, 1994). A similar spread of *Salmonella* to vulnerable newly-hatched chicks is seen with naturally-infected eggs in hatchers (Bailey *et al.*, 1994; Cox *et al.*, 1990). For this reason, sanitising treatments are usually applied to the atmosphere in hatchers, with controlled levels of formaldehyde and, to a lesser extent, hydrogen peroxide sometimes proving efficacious (Bailey *et al.*, 1996; Davies and Wray, 1994). Eggshells and fluff from affected hatchers are widely contaminated, and poor control of airborne fluff or careless handling of hatchery waste can contribute to intractable contamination problems (Arts and Meyerhof, 2000; Cox *et al.*, 1990).

Chicks infected in hatchers are highly susceptible to early intestinal colonisation and multiplication, and will excrete enhanced numbers of organisms. Further horizontal transmission will occur when chicks are sorted and during transport in crates, and crate liners are a sensitive sample for the detection of *Salmonella* contamination emanating from hatcheries. Hatchery-associated *Salmonella* are commonly found further down the production chain, in broiler flocks (Bailey *et al.*, 2001; Byrd *et al.*, 1999; Chriél *et al.*, 1999; Christensen *et al.*, 1997; Davies *et al.*, 2001; McCrea *et al.*, 2006; Thorns, 2000) and on broiler carcasses (Bailey *et al.*, 2001; Bailey *et al.*, 2002; Corry *et al.*, 2002; Liljebjelke *et al.*, 2005). These will often be serovars other than *S.* Enteritidis. Hatcheries have been identified as a risk factor for *Salmonella* Typhimurium infection of Danish broiler flocks (Skov *et al.*, 1999). As a single hatchery typically will receive eggs from several flocks and will dispatch chicks to several premises, the role of hatcheries in collecting and disseminating *Salmonella* contamination is potentially large, and may be compounded by back-transfer of contamination to breeder premises on equipment or personnel (Davies *et al.*, 1997).

3.4. Impact of *Salmonella* prevalence in breeding hens on *Salmonella* prevalence in broilers and layer flocks

Only a small number of *Salmonella* serovars are considered to be intrinsically invasive. *S.* Gallinarum / Pullorum is a prime example of this but, being host adapted, is not a significant foodborne pathogen. *S.* Enteritidis is relatively effectively egg transmitted. Some strains of certain serovars such as *S.* Typhimurium, *S.* Virchow, *S.* Infantis and *S.* Hadar may also be classified as potentially invasive. The virulence of these strains varies in different countries according to the varying clonal complexes that are circulating. Some strains of certain serovars would also be considered to be potentially more invasive in humans than the regulated serovars. Examples of these are *S.* Choleraesuis, *S.* Dublin, *S.* Thompson, *S.* Berta, but there are many other serovars that are more likely to be invasive, i.e. produce a higher proportion of systemic infections, than *S.* Typhimurium or *S.* Enteritidis. Although any *Salmonella* serovar can pass through a hatchery to infect progeny, many are relatively insignificant in terms of human infection despite being frequently found in incidents from broiler flocks. These serovars appear to be adapted to the environment of feed mills, hatcheries or poultry houses so although they may be difficult to eradicate from these situations they often do not represent a significant public health risk.

In the world-wide published literature, there are a modest number of prospective studies examining *Salmonella* types (usually serovars) at several stages of integrated broiler operations. Corry *et al.* (2002) examined two UK companies, noting that for one company all six serovars found in its hatchery (S. Binza, *S.* Enteritidis PT6, *S.* New Brunswick, *S.* Senftenberg, *S.* Typhimurium DT99 and *S.* Virchow) were also found in its abattoir, and for the other company, two hatchery serovars (*S.* Enteritidis PT4, *S.* Mbandaka) were found in its abattoir and two (*S.* Livingstone, *S.* Thomasville) were not. Persistent contaminants of both company feedmills were the serovars most frequently found in the respective abattoirs. Further subtyping of isolates from this study (Liebana *et al.*, 2002) by combined plasmid profiling, ribotyping and pulsed-field gel electrophoresis (PFGE) showed a *S.* Enteritidis strain to be common to the hatchery and a broiler flock, although not identical in this scheme to strains from the abattoir. Plasmid types of *S.* Binza were common to hatchery, broiler house and feedmill. The tendency of *Salmonella* serovars to diversify *in situ* (Brown *et al.*, 1992) may reduce the power of highly discriminatory typing schemes to trace strains through a series of stages.

An investigation into an increased incidence of *S.* Tennessee in Danish broiler flocks revealed a hatchery in which the serovar had become established in hatchers (Christensen *et al.*, 1997). Plasmid analyses and ribotyping showed hatchery and broiler strains to be highly similar and enhanced cleaning and disinfection, resulting in elimination of the serovar from the hatchery, was followed by its decline in broiler flocks.

A study in the USA examined four consecutive broiler flocks on each of two farms, with ribotyping of isolates (McCrea *et al.*, 2006). Of seven serovars isolated, *S.* Kentucky was heavily predominant, uniform in respect of the ribotyping, and was found in samples from hatchery eggshells and dead day-old chicks, through the grow-out period, and on processed broiler carcasses. It was not found in disinfected houses prior to placement of flocks in the early part of the study, but was isolated infrequently from feed equipment, an environmental swab and standing water later in the life of the flocks.

A multistate study of four integrators in the USA (Bailey *et al.*, 2001) examined samples from hatcher basket liners through to slaughter. Of 36 serovars isolated, 12 were found on processed carcasses, and nine of these were also found on hatcher basket liners. *S.* Thompson was common in liners, on farms and on broiler carcasses. Other serovars found on hatcher basket liners and carcasses were: *S.* Brandenburg, *S.* Infantis, *S.* Kentucky, *S.* Mbandaka, *S.* Montevideo, *S.* Senftenberg, *S.* Typhimurium and a variant of *S.* Typhimurium, 4,5,12:i-monophasic. Serovars found in feed were not frequently found on carcasses.

One integrator with known *Salmonella* problems in the USA was found to have indistinguishable *Xba*1 PFGE subtypes of both *S.* Enteritidis and *S.* Typhimurium in its breeder farm, hatchery, broiler flocks and processed carcasses (Liljebjelke *et al.*, 2005). In the same company, *S.* Kentucky was found in the breeders, broiler flocks and carcasses (but not hatchery material), and an indistinguishable PFGE type of *S.* Heidelberg was traced from hatcher basket liners to carcasses.

Another study in the USA in a single company (Bailey *et al.*, 2002) showed no link between the several *Salmonella* serovars isolated, at low frequency, from two breeder flocks (*S.* Thompson, *S.* Kentucky, *S.* Java, *S.* Tennessee) and isolates from subsequent stages of the operation. A peak incidence of positive samples (98%) was obtained from hatchery material (fluff, eggshells, chick faeces and carcass rinses), and the serovars found at this stage (*S.* Senftenberg and *S.* Ohio) were also found in subsequent production flocks and on processed

carcasses. Three other serovars found on carcasses were previously isolated in production flocks or at the abattoir.

Two integrated companies were studied in Korea (Kim *et al.*, 2007), with *S.* Enteritidis of identical *Xba*1 PFGE patterns being found in breeding flocks, production flocks and processed carcasses in one instance, and in hatcheries, production flocks and carcasses in two other instances. *S.* Heidelberg was found on a breeder premises, in the hatchery and on carcasses in one company.

Less data are available in the case of laying hens but transmission of *S*. Enteritidis PT6 from a layer breeding company to commercial laying flocks via the hatchery was demonstrated in UK by field investigations and molecular typing (Davies *et al* 2003). The international dissemination of *S*. Enteritidis during the 1980s is thought to have been promoted by international trade in breeding chickens before monitoring programmes were in place (Evans *et al* 1999), with evidence of *S*. Enteritidis infection of primary breeding flocks, possibly originally acquired from feed, occurring sporadically in various parts of the world (O'Brien 1990, Nakamura *et al* 1993, Edel, 1994).

In several countries vaccination against *S*. Enteritidis has been used in parent breeders and commercial laying hens for many years. 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 vertical transmission and the within flock-prevalence as well as numbers of organisms excreted. Inactivated injectable vaccines may lead to maternal antibodies being transmitted in eggs that may influence the establishment of early infection in chicks placed in a contaminated environment (Inoue *et al*, 2008). It is therefore possible that the limited data on the transmission of *Salmonella* from parent flocks to laying hens may result from reduced detection associated with vaccination. The intensity of monitoring per holding and older age of the birds is also likely to influence the detection of *Salmonella* in laying flocks compared with commercial broiler flocks. Further details on the use of vaccines for the control of *Salmonella* in poultry have been discussed in a previous EFSA opinion (EFSA, 2004c)

In conclusion, serovars other than *S*. Enteritidis and *S*. Typhimurium and the other regulated serovars may be associated with pseudo-vertical transmission. However, true vertical transmission seems to be associated with particular strains of *S*. Enteritidis.

4. Analysis of the *Salmonella* serovar distribution and prevalence in breeders and production flocks in the EU

Results of the monitoring for *Salmonella* in breeding flocks of *Gallus gallus* in the EU for 2007 are presented in table 6.

Table 6. *Salmonella* in breeding flocks of *Gallus gallus* (all types of breeding flocks, flock-based data) during production period in countries running control programmes in accordance with Regulation (EC) No 2160/2003 (source: EFSA, 2009).

				Breeding flocks (elite, grand parent and parent) % positive								
Country	**Period**	**Sampling unit**	**N**	**% pos (all)**	**5 target serovars**	**S. Enteritidis**	**S Typhimurium**	**S. Infantis**	**S. Virchow**	**S. Hadar**	**Other serovars**
Austria	production	flock	61	6.6	0	0	0	0	0	0	6.6
Belgium	production	flock	498	3.8	1.2	0.2	0.6	0	0.4	0	2.6
Bulgaria	production	flock	260	0	0	0	0	0	0	0	0
Czech Republic	production	flock	552	7.1	5.1	4.3	0.5	0.2	0	0	2.0
Cyprus	nr	flock	19	26.3	5.3	5.3	0	0	0	0	21
Denmark	production	flock	270	1.1	1.1	0	1.1	0	0	0	0
Estonia	production	flock	3	0	0	0	0	0	0	0	0
Finland	production	flock	170	0	0	0	0	0	0	0	0
France	production	flock	1,177	0.6	0.6	0.3	0	0.1	0	0.2	0
Germany	production	flock	4,155	1.0	0.1	0.1	<0.1	0	0	0	0.8
Greece	production	flock	38	13.2	13.2	5.3	0	0	0	7.9	0
Hungary	production	flock	2,164	1.2	0.9	0.4	0.1	0.4	0	0	0
Ireland	production	flock	489	5.5	0	0	0	0	0	0	5.5
Italy[1]	nr	flock	391	2.3	1.5	0.3	0.3	0	0.3	0.8	0.8
Latvia	production	flock	21	0	0	0	0	0	0	0	0
Lithuania	nr	flock	62	3.2	0	0	0	0	0	0	3.2
Netherlands	production	flock	1,172	1.3	0.9	0.8	0	0.1	0.1	0	0.3
Poland[1]	Production	flock	965	3.2	3.2	2	0.6	[2]	[2]	[2]	0
Portugal	production	flock	117	15.4	15.4	13.7	0	0.9	0.9	0	0
Romania	production	flock	24	4.2	4.2	4.2	0	0	0	0	0
Slovakia	production	flock	597	1.2	1.0	1.0	0	0	0	0	0.2
Slovenia	production	flock	118	0	0	0	0	0	0	0	0
Spain	production	flock	855	3.4	2.3	1.4	0.4	0	0.1	0.5	1.2
Sweden	production	flock	138	0.7	0.7	0	0.7	0	0	0	0
United Kingdom	production	flock	1,633	1.9	0.1	0	0.1	0	0	0	1.8
EU Total			**15,949**	**2.9**	**1.4**	**1.0**	**0.2**	**0.1**	**0.1**	**0.1**	**1.3**

nr=details not reported

1. Italy and Poland unspecified the type of flocks. For those countries, N may thus not only include flocks in the production period but also day-old chicks or rearing period flocks.

2. Poland reported only five serovars with regard to breeding flocks of *Gallus gallus* within the *Salmonella* control programme.

Out of these data, it can be seen that 15 out of 25 MS met the target according to Regulation (EC) No 1003/2005, with additionally 2 MSs very close to the target. Furthermore, 8 out of 25 MS already achieved a flock prevalence of 1% or less for all serovars, with 4 very close (assuming that all flocks in which any serovar has been found have been accurately reported). Note, however, that Table 6 includes data on elite and grandparent flocks, which may have influenced the prevalence towards lower values, and some MSs have a small number of breeding flocks. It is clear that infection with *S*. Enteritidis still predominated in breeding flocks in 2007 so improved control of this serovar should remain a priority.

An analysis of *Salmonella* monitoring and prevalence figures in poultry (*Gallus gallus*) in the European Union between 2004-2007 has been carried out, and is presented in the annex to this opinion. Thus, the annex has to be read as part of this opinion, and in particular as part of this section.

In the annex, data analysis was carried out in the context of this mandate of both the distribution and prevalence of the different *Salmonella* serovars in parent breeders and production flocks in the EU, based on data originated from that reported by all MSs (excluding Malta and Rumania), plus Norway in the framework of Directive 2003/99/EC Regulation (EC) 2160/2003 and Regulation (EC) 1003/2005. GB data for the period 2000 to 2008 were also analysed, as an example from an individual Member State where more data were available.

In most cases, the descriptive data analysis did not find indications of differing proportion positive flocks between the breeding and production stages, by line of production – based on the regular monitoring results. This can be explained firstly by the fact that some MSs have few flocks and or positive flocks (rare phenomena below or around 1%). An exception was the comparison between the proportion of positive flocks based on the regular monitoring results and the prevalence estimates of the baseline survey figures. Clearly most MSs had productive flocks being substantially more positive covered by the latter figures. This may be explained by the more sensitive sampling design applied in the baseline surveys.

Several detailed points presented in the annex addressed some issues related to data comparability and reliability. These include:

(a) Regulatory requirement constrains, as serovar reporting beyond legal requirements both for breeding and production flocks;

(b) Statistical applicability constrains due to the design of the data collection strategies;

(c) Intra and extra Community movement of poultry, which even if regulated could still interfere in the within member correlation of prevalence levels and serovars in the different flocks;

(d) The novel but not yet fully harmonised monitoring regime in breeding flocks in the EU. There are some differences between MSs in the detailed implementation of monitoring programmes which may result in different sensitivities in detecting and reporting positive flocks. This is most evident for the practice of applying confirmatory tests after positive results found during official controls by some MSs, sometimes with less sensitive sampling schemes. Since results are only reported after confirmation, this may result in biased reporting for the regulated *Salmonella* serovars.

Thus, any attempt to statistically analysing these data and inferring conclusions on the impact of prevalence values in parent breeding flocks to production lines would have limited scientific validity and might produce biased results. Furthermore, correlation analyses are based on sets of data from which neither mechanistic nor biological correlations can be inferred. It is expected that the current harmonised protocols for monitoring of *Salmonella* in breeding hens, and the forthcoming ones for laying hens and broilers, should provide a better database for analysis in future years. It is therefore recommended that a further consideration of the relationship between breeding and production flocks be carried out when harmonised data from control programmes in each sector is available. Such analysis should also be supported by modelling.

Beyond these limitations, the different analysis performed showed some degree of temporal correlation between serovar occurrence in breeding and production lines. Moreover, this correlation was stronger for *S*. Enteritidis and *S.* Typhimurium than for the other targeted serovars. Nevertheless, the results were not consistent between the different types of analysis performed.

5. Mathematical models

Several risk assessment studies have been published on *Salmonella* in the egg production chain. However, most models consider only stages from primary production onwards and do not consider the impacts of the breeding pyramid on the infection of layers. These will not be further discussed in here.

Nauta and colleagues, present a model for transmission of *Salmonella* through the poultry breeding pyramid, from grandparent stock to broiler slaughter houses (Nauta *et al.*, 2000). Further details are included in Appendix C.

Transmission may be dependent on the contamination in the previous production stage (vertical transmission) or may be independent thereof (horizontal transmission). Parameter estimates are based on expert elicitation on *Salmonella* prevalence in different production stages, referring to the situation in the Netherlands in the late nineteen-nineties. Applying the model to other situations assumes that the model parameter values do not change across a broad range of input levels. It is only valid up to a prevalence in the breeding hens of approximately 45%. The model does not differentiate between *Salmonella* serovars. It is used to describe the baseline situation, and to assess the effects of different intervention strategies. Model results suggest that even a reduction from a relatively low prevalence in the breeding hens to the theoretical target of 1% may result in an appreciable reduction of the prevalence in broilers (see Appendix D for details).

Care should be taken to extrapolate these results to the current situation in the EU, as there will be significant differences in the structure of the chains, in prevailing control strategies and in the relative importance of contamination routes. Further model development, and focussed data collection, would be needed to further substantiate these preliminary conclusions.

Ranta and Maijala (2002) present a model on *Salmonella* transmission in the broiler production chain, from grandparents to broilers. The model is used to estimate the true flock prevalence, and the probabilities of horizontal and vertical transmission based on surveillance data, and to assess the effects of intervention methods. The model does not differentiate between *Salmonella* serovars. This model was adapted for the egg production chain by Lievonen *et al.*, 2006. The model describes the egg production chain from primary production to consumption and consumer health risks. The Primary Production Inference Module includes a link between a breeder flock inference model and a production flock inference model. The risk assessment concerns S. Enteritidis only.

In conclusion, some risk assessment models are available to quantitatively analyse the relationship between *Salmonella* infection in breeder flocks and in production flocks but the parameter estimates do not differentiate between *Salmonella* serovars, or concern *S.* Enteritidis only. Hence, they are not informative for the current question but could be used for further analysis if serovar-specific parameter estimates become available.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

General Conclusions

- Salmonellosis is the second most commonly reported foodborne disease in the European Union as a whole, even though the notification rate has significantly decreased since 2004. However, in individual Member States both increasing and decreasing trends in notification can be observed.
- As previously addressed by EFSA, any serovar that is not animal host-adapted is considered capable of causing gastro-intestinal illness of varying severity in humans and thus should be considered of public health significance. Nevertheless, there are differences between serovars in relationship to their frequency in human illness and association with particular food chains that may affect food safety decision making.
- *Salmonella* Enteritidis and *Salmonella* Typhimurium have been the serovars most frequently associated with human illness in the EU in 2006 and 2007. Together, they account for more than 80% of all the isolates to which typing was applied. Other serovars, including those specified in Regulation (EC) No 1003/2005 (*Salmonella* Hadar, *Salmonella* Infantis and *Salmonella* Virchow), did not individually exceed 1%.
- Attribution models from two Member States, and outbreak data from the EU and elsewhere, suggest that in relation to meat and eggs from *Gallus gallus*, *Salmonella* Enteritidis is the serovar most frequently associated with human illness.
- *Salmonella* Enteritidis is particularly associated with commercial layer flocks. In broilers and broiler meat, the reported serovar distribution is highly variable between Member States, with serovars not frequently associated with human illness predominating in some (clusters of) Member States. However, this situation is dynamic and some of these serovars have recently emerged in human cases.
- In general, *Salmonella* serovars may differ significantly in their human pathogenic potential. There are indications that beside *Salmonella* Enteritidis and *Salmonella* Typhimurium certain serovars, including *Salmonella* Dublin, *Salmonella* Virchow, *Salmonella* Heidelberg and *Salmonella* Choleraesuis are more likely to be invasive for humans and their clinical course in humans more severe.
- Resistance against antimicrobials of critical importance in human medicine is observed more frequently than in other serovars in *Salmonella* Enteritidis and *Salmonella* Typhimurium and certain other serovars, including *Salmonella* Paratyphi-B, *Salmonella* Hadar, *Salmonella* Virchow, *Salmonella* Heidelberg, *Salmonella* Newport and *Salmonella* Infantis.
- The poultry production chain has a pyramidal structure and there is considerable potential for transmission of infection from parents to progeny. The import and intra community trade in hatching eggs and day old chicks for parent rearing of production lines could present a risk of dissemination of *Salmonella* beyond the national boundaries, but its control is subject to Community regulations.

- The extent of vertical transmission depends on the ability of individual *Salmonella* strains to colonise the reproductive tract of breeding hens and to survive and multiply in eggs, which varies between and within serovars and phage types.
- Transmission can also occur if *Salmonella* is introduced into a hatchery on contaminated eggs or egg collection equipment. Production animals can also become infected from other animals, feed, transport materials, etc. The extent of such pseudo-vertical and horizontal transmission depends on biosecurity and hygiene measures applied on the farm and in the hatchery.
- Vertical transmission of invasive serovars, especially some strains of *Salmonella* Enteritidis, which have a special ability to colonise avian reproductive tissue is an important issue for breeding flocks but the relevance of other serovars that may occur sporadically and transiently in breeding flocks is less certain as often there is no evidence of transmission to the hatchery.
- The analysis of the EU monitoring data conformed with literature data on a relationship between the occurrence of *Salmonella* between breeding and commercial flocks, in particular for *Salmonella* Enteritidis. However, data availability and quality limited the strength of these conclusions.
- The likelihood of detection of *Salmonella* in breeding flocks is increased by the new sensitive monitoring programme in holdings and hatcheries introduced in 2007.
- A majority of Member States met the current target of 1% or less of flocks of breeding hens remaining positive for five serovars in the year 2007. Approximately half of all MS achieved or approximated this target for all serovars.

Answer to the Terms of Reference

The Terms of Reference could be presented as two separate questions:

1. Assessment of the relative impact on the prevalence of *Salmonella* in flocks of broilers and laying hens if a new target for reduction of *Salmonella* is set in breeding hens being 1% or less flocks remaining positive for all *Salmonella* serovars with public health significance, compared to the theoretical prevalence at the end of the transitional period (1% of five serovars).

Any serovar that is not animal host-adapted is considered capable of causing gastro-intestinal illness of varying severity in humans. In the EU, two serovars (*Salmonella* Enteritidis and *Salmonella* Typhimurium) are considered as of paramount public health significance. *Salmonella* Enteritidis is also the serovar most frequently associated with illness related to broilers and broiler meat, as well as with laying hens and eggs. These, as well as other invasive serovars (*Salmonella* Dublin, *Salmonella* Virchow, *Salmonella* Heidelberg and *Salmonella* Choleraesuis), are associated with serious human illness and increased mortality. Antimicrobial resistance is particularly associated with *Salmonella* Typhimurium, but also with several other serovars s including *Salmonella* Enteritidis, *Salmonella* Paratyphi-B, *Salmonella* Hadar, *Salmonella* Virchow, *Salmonella* Heidelberg, *Salmonella* Newport and *Salmonella* Infantis.

The ability of *Salmonella* serovars for vertical transmission is what determines their importance in breeding flocks. *Salmonella* Enteritidis has the greatest potential for true

vertical transmission from breeding hens to their progeny in the broiler meat and egg layer chains via internally contaminated eggs. *Salmonella* Typhimurium is largely transmitted by pseudo-vertical and horizontal means. EU wide control measures for these serovars in breeding hens are expected to contribute to the control of *Salmonella* infections in production stock, and to reduce human health risks from poultry.

The marginal benefits of additional EU-wide control for other serovars in breeders are relatively small: they are currently less frequently associated with human illness and have less potential for vertical transmission. Biosecurity measures applied to control *Salmonella* Enteritidis and *Salmonella* Typhimurium would also have a beneficial effect to control horizontal transmission of other serovars by contaminated feed, resident contamination in hatcheries and farms and spread of infection by movement of personnel, wild animals, equipment and other fomites.

2. Assessment of the relative impact on the prevalence of *Salmonella* in flocks of broilers and laying hens if a new target for reduction of *Salmonella* is set in breeding hens being 1% or less flocks remaining positive for all *Salmonella* serovars with public health significance, compared to the real prevalence in 2007 to be reported by the Member States.

Available risk assessment models are restricted to two Member States, and refer to earlier situations in which different controls were implemented. Notwithstanding horizontal transmission, there are indications that for those serovars for which vertical transmission is possible, controlling *Salmonella* prevalence to very low levels in breeding flocks is necessary to achieve a low prevalence in production stock. Even though important progress has been made towards harmonised data collection, there is currently insufficient data to quantify the impact of controlling *Salmonella* prevalence in breeders on the prevalence in production stock as harmonised monitoring is not yet established in the production sector.

RECOMMENDATIONS

- Maintaining stringent targets and controls at the EU level for *Salmonella* Enteritidis and *Salmonella* Typhimurium in flocks of breeding hens is recommended.
- Further control policies for other *Salmonella* serovars in breeding hens should be guided by the level of their dissemination into production stock in individual EU MS, and may be considered in national control programs.
- Further consideration of the relationship between breeding and production flocks should be carried out when harmonised data from control programmes in each sector is available. Such consideration should also include the further development of quantitative risk assessment models, taking data for specific serovars into account.

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APPENDICES

APPENDIX A. REPORTING AND VERIFICATION OF HUMAN SALMONELLOSIS OUTBREAKS IN THE EU

Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents1 (Zoonoses Directive) covers the epidemiological investigation and reporting of foodborne outbreaks in the Member States (MSs) of the European Union (EU). Thorough investigation of foodborne outbreaks aims to identify the pathogen, the food vehicle involved, and the factors in the food preparation and handling contributing to the outbreak. The Zoonoses Directive makes provisions for such investigations and for close co-operation between various authorities.

The competent authority of each Member State must provide the Commission with a summary report of the results of the investigations of foodborne outbreaks, which is sent to the European Food Safety Authority (EFSA). Minimum reporting requirements for the foodborne outbreaks are laid down in Annex IV (E) to the Directive. In addition, in accordance with the procedure referred to in Article 12, detailed rules concerning the assessment of the reports, including the format and the minimum information they must include, may be laid down.

The data collection may allow the identification of emerging trends in the causative agents and vehicles in the Community. Data regarding foodborne outbreaks provide important information on the number of humans affected annually and complements the picture of the burden of foodborne disease given by the total number of cases of disease in the Community. The added value concerns especially the information on the causative agent-food vehicle combinations responsible for the foodborne outbreaks. This information is necessary when targeting actions to improve food safety in the Community.

In order to obtain more in-depth information on the foodborne outbreaks, more detailed data may be collected from certain particularly well-investigated single foodborne outbreaks. This information would increase the understanding of the epidemiology of the causative agents and could possibly be used for risk assessments.

The 'Report from the Task Force on Zoonoses Data Collection on harmonising the reporting of foodborne outbreaks through the Community reporting system in accordance with Directive 2003/99/EC' (EFSA Journal (2007) 123, 1-16) describes the new reporting specifications. The report is available on the EFSA website at:

http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178662632770.htm

In 2007 EFSA applied for the first time the new harmonized reporting recommendations with categorisation of possible and verified outbreaks (more detailed data). This categorization is done on the basis of strength of evidence of link between human cases and food source.

Possible outbreaks: all outbreaks that *might* be caused by food to study impact of problem. Only total numbers of outbreaks requested according to main causative agent groups (*Salmonella*, *Campylobacter*, etc);

- An outbreak compatible with descriptive epidemiological evidence only
- Includes also outbreaks where the causative agent is unknown
- Descriptive epidemiological evidence: information linking two or more persons with clinical symptoms consistent with a disease caused by the same (typically foodborne) pathogen, with a possible food vehicle in common. The pathogen may or may not have

been isolated from the human cases. There are some indications that the persons may have consumed the same food, but there is no strong evidence to support this link between the human cases and the food (e.g. no detection of the agent from food)

Verified outbreaks: outbreaks with rather strong evidence that outbreak *was* caused by food. This allows more in-depth analysis of food vehicles and causative agents;

- Outbreaks compatible with descriptive epidemiological evidence + one or two of the following : laboratory detection of the causative agent from implicated food; and/or analytical epidemiological evidence (= cohort or case-control study performed with an outcome of significant association between the cases and food)

More detailed data are to be collected:

- Causative agent (pick list)
- Type of outbreak (household/ general /unknown)
- No of human cases
- No of hospitalisations
- No of deaths
- Foodstuff implicated / vehicle (pick list + free text)
- Evidence for the food implicated (lab. detection/ lab. characterisation/ analytical epidemiology)
- Setting (pick list)
- Place of origin of problem (pick list)
- Origin of foodstuff (domestic/ EU trade/ outside EU)
- Contributory factors (pick list)
- For well investigated outbreaks: detailed description (free text)

Data on verified Salmonellosis outbreaks reported by EU MS in 2007, indicating number of those linked to the consumption of egg (or egg products) and poultry meat (or poultry meat products thereof) are presented below in Table1.

Table 1. Serovar distribution of *Salmonella* isolates from foodborne outbreaks in 2007 linked to consumption of egg or egg products (including raw eggs in bakery products) and poultry meat and products thereof reported by EU MSs (raw data from EFSA, CSR, 2009).

Salmonella serovar	EU Total 2007				Eggs and egg products (including raw eggs in bakery products)				Poultry meat (*Gallus gallus*) and products thereof			
	Outbreaks	Human cases	Hospitalisations	Deaths	Outbreaks	Human cases	Hospitalisations	Deaths	Outbreaks	Human cases	Hospitalisations	Deaths
S. Enteritidis	260	5,009	1,244	9	132	1,645	398	3	11	133	32	0
S. Typhimurium	55	954	179	0	18	97	27	0	1	71	4	0
S. Agona	2	40	1	0	0	0	0	0	0	0	0	0
S. Anatum	2	31	0	0	0	0	0	0	0	0	0	0
S. Bovismorbificans	1	15	0	0	0	0	0	0	0	0	0	0
S. Brandenburg	1	2	2	0	0	0	0	0	0	0	0	0
S. Bredeney	2	14	2	0	0	0	0	0	0	0	0	0
S. Coeln	1	3	3	0	0	0	0	0	0	0	0	0
S. group B	1	26	5	0	0	0	0	0	0	0	0	0
S. group D	1	3	3	0	1	3	3	0	0	0	0	0
S. Heidelberg	2	25	7	0	0	0	0	0	1	12	1	0
S. Infantis	1	36	0	0	0	0	0	0	0	0	0	0
S. Kimuenza	1	2	2	0	0	0	0	0	0	0	0	0
S. Newport	2	9	6	0	0	0	0	0	0	0	0	0
S. Panama	1	31	4	0	0	0	0	0	0	0	0	0
S. Senftenberg	1	3	0	0	0	0	0	0	0	0	0	0
S. Stanley	1	51	0	0	0	0	0	0	0	0	0	0
S. Thompson	1	2	0	0	1	2	0	0	0	0	0	0
S. Virchow	2	23	2	0	0	0	0	0	0	0	0	0
S. Weltevreden	2	27	0	0	0	0	0	0	0	0	0	0
Salmonella spp., unspecified	63	881	71	0	23	150	27	0	2	113	3	0
EU total	**403**	**7,187**	**1,531**	**9**	**175**	**1,897**	**455**	**3**	**15**	**329**	**40**	**0**

Note: Spain (N=187) is not included due to reporting aggregated data.

APPENDIX B. PUBLISHED PEER-REVIEWED SCIENTIFIC PAPERS REPORTIN ON HUMAN SALMONELLOSIS OUTBREAKS LINKED TO CONSUMPTION OF EGG AND EGG PRODUCTS

The following parameters were used in a literature search carried out employing PubMed on the 2nd December 2008:

1. salmonella[mh] OR salmonella infections[mh:noexp] OR salmonel*[ti]

2. eggs[mh] OR "egg proteins, dietary"[mh:noexp] OR egg white[mh] OR egg yolk[mh] OR egg*[tiab]

3. disease outbreaks[mh] OR food poisoning[mh:noexp] OR salmonella food poisoning[mh] OR food contamination[mh:noexp] OR food microbiology[mh] OR outbreak*[ti] OR contamination[ti] OR infection*[ti] OR epidemic*[ti] OR epidemiology[ti] OR incidence[ti] OR prevalence[ti]

4. #1 AND #2 AND #3

5. (animals[mh] NOT humans[mh]) OR laying[ti] OR layer[ti] OR hen[ti] OR hens[ti] OR flock*[ti] OR hatcheries[ti] OR chicken*[ti] OR broiler*[ti] OR poultry[ti] OR quails[ti]

6. vaccin*[ti] OR heat resistance[ti] OR pasteurization[ti] OR survival[ti] OR irradiation[ti] OR shelf life[ti] OR inactivation[ti] 07:29:22 213040

7. #4 NOT (#5 OR #6)

These search, after a scrutiny of the abstracts, produced a total of 224 publications as presented in Table 1.

Table 1. Publications reporting on outbreaks of salmonellosis in humans where egg or egg products were identified as the source.

Year	Country	Serovar	N outbreaks/cases (where reported)	Associated Source	Structure	Reference
2005	Italy	Enteritidis	1 outbreak, unknown	Cooked dish with egg		Rizzo, 2006
2006	UK	Enteritidis	1 outbreak, 49 cases	Egg mayonnaise bagel		Morgan *et al.*, 2007
2006	UK	Enteritidis	1 outbreak, 15 cases	Egg containing tiramisu		Calvert *et al.*, 2006
2005	Austria	Enteritidis	1 outbreak, 35 cases	Spätzle (traditional pastalike side dish with egg)		Schmid *et al*., 2007
2005	Australia	Typhimurium	5 outbreaks, 125 cases	Egg products		Stephens *et al.*, 2007
2002-2003	Spain	Typhimurium	11 outbreaks, unknown	Egg and Egg products		Crespo *et al.*, 2005
	Spain	Hadar	11 outbreaks, unkwnow	Egg and Egg products		
2006	Latvia	Enteritidis	1 outbreak, 7 cases	Mayonnaise made with raw eggs		Brila *et al.*, 2006
1973-2001	USA	Heidelberg	3 outbreaks	Eggs		Chittick *et al.*, 2005
			17 outbreaks	Egg contaminated food item		
			8 outbreaks	Eggs and poultry		
2004	Austria	Enteritidis	1 outbreak, 300 cases	Eggs		Much *et al*, 2005
2004	Korea	Enteritidis	1 outbreak	Egg products		Lim *et al*, 2005
1998	USA	Enteritidis	1 outbreak, 38 cases	Eggs		Burr *et al*., 2005
2002	Catalonia	Enteritidis	1 outbreak, 1435 cases	Egg products		Camps, *et al*, 2005
1992-2002	UK	Enteritidis	497 outbreaks	Eggs, egg products		Gillespie *et al*.2005
2003	USA	Enteritidis	1 outbreak, 1 case	Egg product, meringue	cafeteria	Mazurek *et al*., 2003
2003	Spain	Enteritidis	1 outbreak, 250 cases	Egg product	dining hall	Carbo Malonda *et al*., 2005
2003	USA	Typhimurium	18 cases	Egg salad	supermarket	CDC, 2003

Year	Country	Serovar	N outbreaks/cases (where reported)	Associated Source	Structure	Reference
2001	Taiwan	Enteritidis	1 outbreak, 28 cases	Egg product	hospital	Lu *et al*, 2004
2002	Australia	Potsdam	1 outbreak, 12 cases	Egg salad dressing	Restaurant	Unicomb et la., 2003
1999	Brazil	Enteritidis	1 outbreak, 8 cases	Egg-based enteral nutrition	Hospital	Matsuoka *et al*, 2004
2001	Japan	Enteritidis	1 outbreak, 96 cases	Dessert bun, cross contamination with eggs	School	Matsui *et al* 2004
2002	UK	Enteritidis	1 outbreak, 38 cases	Egg fried rice	Chinese restaurant	Badrinath *et al*., 2004
2001	USA	Enteritidis	4 outbreaks, 688 cases	Eggs	Prison	CDC, 2003
2002	Australia	Typhimurium	1 outbreak	Raw egg		Hall, 2002
2000	UK	Indiana	1 outbreak, 17 cases	Mayonnaise	Hospital	Mason *et al*., 2001
2002	Australia	Typhimurium		Raw eggs	Aged care facility	Tribe *et al*., 2002
2000	Australia	Typhimurium	1 outbreak, 53 cases	mock ice-cream dessert	community dinner	Sama *et al*., 2000
2000	UK	Enteritidis	3 outbreaks, 24 cases	Food containing raw egg	Public event	Eijdokun, *et al*, 2000
1997	USA	Enteritidis	17 cases	Cheesecake sligh cooked eggs	Scout troops	
1997	USA	Enteritidis	7 outbreaks, 9 cases	Lasagna	Public event	
1997	USA	Enteritidis	2 outbreaks 91 cases	hollandaise sauce with uncooked eggs	Restaurant	
1999	DK	Enteritidis		Uncooked eggs	New year celebration of the Copenhagen Medical Association	Neimann *et al*, 1999
1996	USA	Enteritidis	7 outbreaks	Chili rellenos (eggs)	Restaurant	Mc Neil *et al*, 1996
1993-97	Brazil	Enteritidis	729 cases	raw or undercooked eggs		Peresi *et al*, 1998
1996	Saudi Arabia	Enteritidis	1 outbreak	Mayonnaise, and cross contam	Restaurant	Al Ahmadi *et al*, 1998
1992	Spain	Enteritidis/ Typhimurium	5 outbreaks, 545 cases	boiled eggs, omelette, soufflé and home-made russian salad	1 school, 2 restaurants, and 1 residence) and 1 at home	Arnedo *et al*, 1998

Year	Country	Serovar	N outbreaks/cases (where reported)	Associated Source	Structure	Reference
1998	UK	Enteritidis	1 outbreak, 4 cases	Raw eggs	Body builders	Mackenzie *et al*, 1998
1993	USA	Enteritidis	3 outbreaks, 38 cases	Egg dish, mayonnaise, egg sauce		
1998	UK	Enteritidis	1 oubreak, 24 cases	home-made ice cream	Private party	Dodhia *et al*, 1998
1994	Mexico	Enteritidis	1 outbreak, 10 cases	Egg product	Hospital's cafeteria	Molina gamboa *et al*, 1997
1989	Japan	Enteritidis	5 outbreaks	Egg-food		Kusunoki *et al*, 1989
1997	UK	Enteritidis	1 outbreaks, 13 cases	egg sandwiches		Holtby *et al*, 1997
1997	UK	Enteritidis	1 outbreak, 17 cases	Mayonnaise	hotel	Doherty *et al*, 1997
1995	UK	Enteritidis	1 outbreak, 36 cases	Marshmallow - raw egg white	bakery	Lewis *et al*, 1996
1995	USA	Enteritidis	7 cases	Turkey meat, eggs	Private home	CDC 1996
1976-94	USA	Enteritidis	582 outbreaks, 24,058 cases	raw shell eggs (i.e., unpasteurized eggs		CDC 1996
1996	UK	Enteritidis	4 outbreaks	Cross contam gateau	Bakery	Wight *et al*, 1996
1994	Usa	Enteritidis	224,000 cases	ice cream (Schwan's		Hennessy *et al*, 1996
1995	UK	Enteritidis	1 outbreak, 4 cases	Rice salad with eggs		Evans *et al*, 1995
1993	Brazil	Enteritidis	1 outbreak, 211 cases	Patè made with fresh eggs		Kaku *et al*, 1995
1994	UK	Enteritidis	1 outbreak	Home-made ice cream		Morgan *et al*, 1994
1985-94	USA	Enteritidis	47 outbreaks, 2279 cases	Eggs		Morse *et al*, 1994
1981-95	USA	Enteritidis	380 outbreaks, 13056 cases	Eggs (82%)		Mishu *et al*, 1994
1991	Ethiopia	Newport	1 outbreak, 6 cases	unpeeled undercooked eggs		Aseffa *et al*, 1994
1991	UK	Enteritidis	1 outbreak, 83 cases	mayonnaise		Irwin *et al*, 1993
1993	USA	Enteritidis	1 outbreak, 690 cases	raw egg based-sauce		Goodman *et al*, 1993

Year	Country	Serovar	N outbreaks/cases (where reported)	Associated Source	Structure	Reference
	UK	Enteritidis	1 outbreak, 17 cases	Custard with fresh shell eggs		Barnes *et al*, 1992
1991	USA	Enteritidis	66 outbreaks	Raw shell eggs		CDC, 1991
1989	UK	Typhimurium	68 cases	mayonnaise		Ortega benito *et al*, 1992
1989	UK	Enteritidis	1 outbreak, 4 cases	egg mayonnaise	restaurant	Ahmed *et al*., 1992
1992	Uk	Enteritidis	1 outbreak, 7 cases	Raw egg product		Harrison *et al*, 1992
1986	Finland	infantis	2 outbreaks, 226 cases	egg sandwiches	catering	Hatakka, 1992
1989	UK	Enteritidis	3 outbreaks	Raw egg		Salmon *et al*. 1991
1990	USA	Enteritidis	3 outbreak	eggs		CDC, 1990
1990	UK	Enteritidis	1 outbreak, 173 cases	egg-based sauces	wedding	Stevens *et al*, 1989
1988	UK	Enteritidis	2 outbreaks, 18 and 84 cases	home-made ice-cream containing uncooked eggs		Cowden *et al*, 1989
1988	UK	Typhimurium	76 cases	mayonnaise		Mitchell *et al*. 1989
1985	USA	Enteritidis	3 outbreaks, 71 cases	Scrambled eggs		Lin *et al*, 1988
1982	USA	Typhimurium	8 cases	homemade ice cream		Taylor *et al*, 1984
1979	USA	Typhimurium	36 cases	Egg based food, salad		Blaser *et al*, 1979
1966-1976	USA	Enteritidis, typhimurium (45%)	22 outbreaks, 292 cases	homemade ice cream		Gunn *et al*, 1978
1973	USA	Typhimurium	32 cases	raw egg beaten in milk ("egg-nog")		Steere *et al*, 1975
1970	USA	Typhimurium	1790 cases	Ice cream		Armstrong *et al*, 1970

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APPENDIX C. PUBLISHED PEER-REVIEWED SCIENTIFIC PAPERS REPORTIN ON HUMAN SALMONELLOSIS OUTBREAKS LINKED TO THE CONSUMPTION OF POULTRY MEAT AND PRODUCTS THEROF

The following parameters were used in a literature search carried out employing PubMed on the 30th January 2009:

1. salmonella[mh] OR salmonella infections[mh:noexp] OR salmonel*[ti]

2. broiler*[tiab] OR chicken*[tiab] OR chickens[mh] OR poultry[ti] OR poultry[mh]

3. meat[tiab] OR meat[mh] OR poultry products[mh] OR meal[tiab] OR fillet*[tiab] OR wings[tiab] OR carcass*[tiab] OR consumption[tiab]

4. disease outbreaks[mh] OR food poisoning[mh:noexp] OR salmonella food poisoning[mh] OR food microbiology[mh] OR outbreak*[ti] OR infection*[ti] OR epidemic*[ti] OR epidemiology[ti] OR incidence[ti]

5. #1 AND #2 AND #3 AND #4

Total number of publications produced 822

These, after a scrutiny of the abstracts, produced a total of 25 publications as presented in Table 1 below.

Table 1. **Publications reporting on outbreaks of salmonellosis in humans where poultry meat or products therof were identified as the source.**

Year	Country	Serovar	N outbreaks/cases (where reported)	Associated Source	Reference
1966	USA	Typhimurium	1/107	Ready-to-eat barbecued chickens	Werner et al., 1969
1968	UK	Virchow	1/160	Spit-roasted Chicken	Semple et al,
1975	USA	Infantis, Agona, Schwarzengrund	1/125	Chicken meat	Levy et al., 1975
1976	Switzerland	Saint-paul	1	Grilled chicken	Pagon, 1976
1986	UK	Typhimurium	1/195	Chicken meat	Glynn and Palmer, 1992
1990	UK	Berta	1	Chicken meat	Threlfall et al, 1992
1992	Peru	Paratyphi	1/159	Chicken meat	Pazzaglia et al, 1992
1994	UK	Virchow	188	Chicken meat	Willocks et al, 1994
1996	Thailand	Enteritidis	1/125	chicken meat	Kantama et al, 1996
1997	Northern Ireland	Bredeney	1/10	Chicken meat	Moore et al, 2003
1997	USA	Heidelberg	1/49	Chicken liver	Layton et al, 1997
1998-2006	USA	Heidelberg	¼	stuffed chicken products	Smith et al., 2008
1998-2006	USA	Enteritidis	1/27	stuffed chicken products	Smith et al., 2008
1998-2006	USA	Typhimurium	2/36	stuffed chicken products	Smith et al., 2008
1998	Australia	Bredeney	1/157	meat or chicken product	Baker et al., 1998
1998	Australia	Typhimurium	1/10	chicken nuggets	Kenny et al., 1999
2000	UK	Enteritidis	70	chicken dishes from Chinese restaurant	Cowden et al, 2003

Year	Country	Serovar	N outbreaks/cases (where reported)	Associated Source	Reference
2001	Greece	Enteritidis	1/303	chicken	Guerin et al, 2006
2002	Greece	Enteritidis	1/164	chicken	Guerin et al, 2006
2002	Austria	Enteritidis	1/30	Chicken meat	Berghold et al, 2004
2003	Greece	Enteritidis	1/199	chicken	Guerin et al, 2006
2003	USA	Enteritidis	1/182	chicken	Kimura et al., 2004
2005	Spain	Hadar	1/2138	Precooked roast chicken	Lenglet A, 2005
2006	Australia	Typhimurium	1/61	Chicken meat	McPherson et al,
2006	USA	Heidelberg	25	chicken	Chittick et al., 2006
2007	USA	Montevideo	964	live poultry	CDC, 2009

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APPENDIX D. A MATHEMATICAL MODEL FOR VERTICAL TRANSMISSION OF SALMONELLA SPP. IN THE BROILER PRODUCTION CHAIN

The model by Nauta *et al*. (2000) was constructed to support decision making on control strategies of *Salmonella* in the broiler production chain in the Netherlands, in the late nineteen-nineties. In that period, *S*. Enteritidis was the most frequently detected serovar in Dutch broiler production. For example, in 1999, 20.4% of broiler flocks were tested positive for *Salmonella* spp., with 5.3% *S.* Enteritidis and 1.3% *S.* Typhimurium (Valkenburgh *et al*., 2000). A control plan had been implemented since 1997. The plan implied strict hygiene requirements (including cleaning and disinfection of empty houses) in all stages of the breeding pyramid, and mandatory testing for *Salmonella* of both incoming and outgoing flocks. Specific measures were implemented for flocks tested positive for *Salmonella*, depending on their position in the breeding chain. For example, breeder flocks where *S.* Enteritidis or *S.* Typhimurium was detected were stamped out. Vaccination was used only in specific situations, such as in flocks which were transferred to farms with a documented history of infection with *S.* Enteritidis.

The model describes the poultry production chain as a chain of consecutive production stages, from grandparent stock to slaughterhouses. Here, we focus on the links parent stock – commercial hatcheries – broiler stock. The model assumes that throughout the chain, the prevalence of Salmonella infection can only increase, there is no reduction from one chain to the other (i.e. in the baseline situation, no controls are implemented). The prevalence in a specific step t of the chain Q_t depends on the prevalence in the previous step (R_t, dependent transmission), and contamination from other sources at the step (P_t, independent transmission).

Then, $1 - Q_t = (1 - P_t)(1 - R_t)$.

The dependent route of transmission is assumed to result from two mechanisms: cross-contamination between flocks at the farm or during transport and composing progeny from multiple parent flocks. The increase of prevalence from step t-1 to step t is modelled by the parameter k_t:

$R_t = (k_t + 1)Q_{t-1}$, and $Q_t = 1 - (1 - P_t)(1 - (k_t + 1)Q_{t-1})$.

The model parameters were estimated from expert elicitation on *Salmonella* prevalence in different production stages, referring to the situation in the Netherlands in the late nineteen-nineties. At that time, the Salmonella prevalence in the parent stock was estimated to be 15%. Using the model of Nauta *et al.* (2000) with other input values for the parents shows the following results (Table 1).

Table 1. **Results of modelled prevalence in hatcheries and broilers, depending on different initial input values of prevalence in parent flocks, employing model from Nauta *et al* (2000).**

Prevalence in parents (%)	Prevalence in hatcheries (%)	Prevalence in broilers (%)
32	65.6	76.6
16	32.8	39.2
8	16.4	20.6
4	8.2	11.2
2	4.1	6.6
1	2.1	4.2
0.5	1.0	3.1

Hence, given the model assumptions, every reduction of prevalence in the breeding hens to the theoretical target of 1% is expected to result in an appreciable reduction of the prevalence in broilers and more stringent targets (*e.g.* 0.5%) would result in still lower prevalences. It must be stressed, however, that demonstrating the achievement of such targets would require large sample numbers.

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