

**Advisory Committee on the
Microbiological Safety of Food**

***Ad Hoc* Group on Eggs**

**An update on the microbiological risk from shell
eggs and their products**

**Advises the Food Standards Agency on the
Microbiological Safety of Food**

Ad Hoc ACMSF Group on eggs

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Terms of Reference

The remit of the group is: -

- To assess the current level of microbiological risk to consumers (including vulnerable groups) from raw or lightly cooked shell eggs and their products.
- To assess how the risk with respect to *Salmonella* has changed since the last ACMSF report on this subject in 2001.

The working group will report back regularly to the ACMSF.

Scope:

- All commercially¹ available edible shell eggs and liquid and frozen eggs including those on retail sale and from catering establishments.
- Shell eggs and liquid and frozen eggs produced in the UK (those from quality assurance schemes and others)
- Shell eggs and liquid and frozen eggs produced in the EU and those imported from third countries.

Outputs:

- The working group will prepare an assessment of the current microbiological risk from shell eggs and liquid and frozen eggs and will also indicate whether the risk associated with *Salmonella* has changed since the ACMSF 2001 report.
- The working group will prepare a report on its findings concerning the current level of risk from shell eggs and liquid and frozen eggs and make a recommendation(s) on whether the Agency's existing guidance on shell eggs remains appropriate. Any other relevant recommendations can be included in this report.
- The working group will present its report to the main ACMSF Committee for endorsement.

¹ Regulation 852/2004 provides the following exemption: "the direct supply, by the producer, of small quantities of primary products to the final consumer or to local retail establishments directly supplying the final consumer"

In order for this exemption to apply, the quantities involved must be small and the supply must be direct to the final consumer.

Under an exemption in EU Egg Marketing Regulations, eggs in shell may be sold directly to the final consumer without any quality or weight grading. Furthermore, the direct supply of small quantities of eggs to the final consumer is also exempt from complying with the food hygiene regulations. FSA guidance suggests up to 360 eggs a week is a small quantity.

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Overall risk assessment and key recommendation

The ACMSF *Ad hoc* Working Group on eggs was asked to assess the current level of microbiological risk to consumers (including vulnerable groups) from raw or lightly cooked shell eggs and their products, and specifically to assess how the risk with respect to *Salmonella* has changed since the last ACMSF report on this subject in 2001.

The Group concluded that with respect to hen shell eggs, whilst a range of micro-organisms could potentially contaminate the shell surface and possibly the egg contents, the only group of organisms of significant importance in respect of contents contamination is *Salmonella*. This latter risk is generally limited to a subset of these bacteria, principally *Salmonella* Enteritidis.

It was the strong view of the Working Group that there has been a major reduction in the microbiological risk from *Salmonella* in UK hen shell eggs since the 2001 ACMSF report. This is especially the case for those eggs produced under the Lion Code, which comprises a suite of measures including: vaccination, a cool chain from farm to retail outlets, enhanced testing for *Salmonella*, improved farm hygiene, better rodent control, independent auditing, date stamping on the eggs and traceability. The risk from imported eggs has also been reduced but not to the same extent. Accordingly, the group suggests that the risk level for UK hen shell eggs produced under the Lion code, or produced under demonstrably-equivalent comprehensive schemes, should be 'VERY LOW', whilst for other shell eggs the risk level should be considered 'LOW'.

In practical terms, the Group considered that the 'VERY LOW' risk level means that eggs produced under the Lion code, or produced under demonstrably equivalent comprehensive schemes, can be served raw or lightly cooked to all groups in society, including those that are more vulnerable to infection, in both domestic and commercial settings, including care homes and hospitals.

The Group considered that for those eggs in the 'LOW' risk group (UK produced non-Lion code hen shell eggs, and non-UK hen shell eggs) that the existing advice should remain i.e. that the very young, the elderly, pregnant women, and those who are already unwell, should not consume these eggs raw or lightly cooked. Similarly, we also consider that existing advice should remain for non-hen shell eggs.

Consumers and caterers must continue to be aware of the need to store eggs properly, to observe use by dates, and to avoid cross-contamination of eggs within the kitchen environment, particularly where the eggs' contents will be consumed raw or lightly cooked.

The Group considered that commercial egg products should continue to be pasteurised.

Key recommendation

The Group recommends that the Food Standards Agency considers amending its advice on eggs in the light of the above.

Chapter 1: Introduction

Background to the review

- 1.1 In March 1991, the Advisory Committee on the Microbiological Safety of Food (ACMSF) set up a sub-group to consider the extent to which eggs were responsible for the incidence of foodborne disease due to the bacteria *Salmonella* (primarily *Salmonella* Enteritidis which was the cause of most outbreaks). A Department of Health (DH) funded survey of the prevalence of *Salmonella* contamination of eggs from retail outlets in the high street in 1991 showed that *Salmonella* were isolated from 65 out of 7045 boxes of six eggs (0.92%). A follow-up DH funded survey in 1995/96 demonstrated that the situation had not improved; *Salmonella* was isolated from 138 of 13970 samples of six eggs (0.99%), despite extensive measures adopted by industry to address the problem.
- 1.2 Given that there was no obvious explanation for the lack of improvement relating to the prevalence of *Salmonella* contamination of UK eggs between 1991 and 1995/96, the Committee set up a second sub-group in 1998 to consider the factors which determine the presence of *Salmonella* contamination in or on eggs. The Committee looked at *Salmonella* infections in humans and the evidence that eggs have a role in human salmonellosis. It also assessed existing measures to reduce *Salmonella* contamination of eggs, the contribution of vaccination and competitive exclusion, and the storage, handling and use of eggs (ACMSF, 2001).
- 1.3 At the time of the Committee's 2001 report, insufficient data were available for the Committee to quantify the risk of *Salmonella* infection from the consumption of raw and/or lightly cooked shell eggs. The Committee did consider a risk assessment model developed by DH with input from members of the working group and concluded that more empirical data were required to support further development of such a model. Since then, more information has become available, particularly on *Salmonella* in laying flocks and prevalence of *Salmonella* contamination in UK and non-UK eggs. The Agency has used some of these data to populate and further develop an exposure assessment model for *Salmonella* and eggs (ACM/937).
- 1.4 The Chief Medical Officer and the Agency have previously highlighted the risk associated with eating raw and lightly cooked eggs and issued public health advice on the safe handling and use of eggs. The Agency's advice historically has always been that "eating raw eggs, eggs with runny yolks or any food that is uncooked or only lightly cooked and contains raw eggs may cause food poisoning, especially in 'at risk' groups such as pregnant women, the elderly

and anyone who is unwell or immuno-compromised. This is because eggs may contain *Salmonella* bacteria which can cause serious illness. People who are not in vulnerable groups who eat soft-boiled eggs or foods containing lightly cooked eggs should not experience any health problems, but cooking eggs thoroughly is the safest option if you are concerned about food poisoning.” A link to the full advice can be found at:

<http://www.nhs.uk/Livewell/Goodfood/Pages/eggs-nutrition.aspx>. Advice concerning the risk associated with eating raw and lightly cooked eggs was developed when reported human *Salmonella* Enteritidis infections were significantly higher than they are now. Although outbreaks linked to eggs continue to occur these are much less frequent than in the 1990s and human cases are predominantly associated with non-UK eggs.

- 1.5 The Agency therefore now considered it appropriate to review its existing advice to determine whether or not it remains applicable and proportionate. In January 2015, the issue was discussed at a plenary meeting of the ACMSF (ACM/1166) and the Committee agreed that it would be appropriate to establish a sub-group to examine the subject more closely.
- 1.6 The ACMSF *Ad Hoc* Group on Eggs was formed in February 2015. The purpose of the group was to assess the current level of microbiological risk to consumers (including vulnerable groups, i.e. the very young, the very old, the pregnant or the immunocompromised) from raw or lightly cooked shell eggs and their products and to assess how the risk with respect to *Salmonella* has changed since the last ACMSF report on this subject in 2001. The remit of this group focussed on all commercially available edible shell eggs and liquid and frozen eggs including those on retail sale and used in catering establishments, these products produced in the UK (those from quality assurance schemes and others) and shell eggs and liquid and frozen eggs produced in the EU and those imported from third countries. Somewhat differently from previous sub-groups, this group was also tasked with reviewing other microbiological hazards that may be present in eggs and egg products; although *Salmonella*, and in particular *Salmonella* Enteritidis, was still the main focus.

***Salmonella* Enteritidis contamination of eggs**

- 1.7 Foodborne disease outbreaks caused by *Salmonella* have been associated with a variety of foods. However, outbreaks caused by *Salmonella* Enteritidis tend to be predominantly associated with eggs and egg products (Doorduyn et al. 2006, Drociuk et al. 2003, Gillespie et al. 2005, Hayes et al. 1999, Mishu et al. 1994, Mølbak and Neimann 2002, Schmid et al., 1996).

- 1.8 Raw shell eggs may become contaminated with *Salmonella* in different ways. The outside of the egg may be contaminated by faeces after laying (Humphrey, Martin, and Whitehead, 1994, and Humphrey 1994), or be internally contaminated with *Salmonella* during laying if the reproductive tract is colonised by the organism. *Salmonella* on the outside of the egg can occasionally migrate through the porous shell to the interior, particularly when eggs are newly-laid or under humid conditions (De Buck *et al*, 2004) but this is thought to be unusual in real life situations, as opposed to laboratory studies. Contamination of the egg contents can also result from infection of the reproductive tissue prior to egg development (de Buck *et al*. 2004, Humphrey 1994). *Salmonella* bacteria can survive in lightly cooked eggs or raw egg dishes and cause human disease (Humphrey *et al*. 1989, 1990).
- 1.9 A number of other, less significant potentially vertically transmitted zoonotic micro-organisms (viruses and bacteria) can be associated with shell eggs and egg products e.g. avian influenza virus, *Campylobacter*, *Escherichia coli*, *Listeria*. Viruses include: Avian influenza virus, Leucosis/sarcoma group of avian type C viruses, Reticuloendotheliosis viruses, and Tremorvirus, Avian encephalomyelitis virus and Avian adenovirus. However, there have been no egg-associated cases of human infection involving the above viral agents in the UK.
- 1.10 Potentially zoonotic egg-associated bacteria include *Campylobacter*, *Escherichia coli* and *Listeria*. Full details and an assessment of the microbiological hazards associated with shell eggs have been carried out in Chapter 3.

A summary of the prevalence of *Salmonella* Enteritidis in chickens and humans

- 1.11 *Salmonella* Enteritidis has been responsible for occasional cases of infection in the UK and elsewhere for over 100 years. However, its emergence as a pandemic chicken meat- and egg-associated global public health problem in the late 1980s caused the largest and most persistent epidemic of foodborne infection attributable to a single subtype of any pathogen. In England and Wales, it is estimated that >525,000 people became ill during the course of the epidemic. The epidemic was associated with the consumption of contaminated chicken meat but more importantly, shell eggs. A decline in numbers of infections started after the introduction of vaccination for *Salmonella* Enteritidis and other control measures in chicken breeding and the production and distribution of eggs and chicken meat (Lane *et. al.*, 2014).

- 1.12 In England and Wales, during the epidemic stage, PT4 accounted for 159 (79%) of 201 egg-associated *Salmonella* Enteritidis outbreaks (Lane et al., 2014). Following interventions by the UK egg industry, largely under the Lion Code, there was a sharp fall in the number and proportion (36/95 or 38%) of egg-associated *Salmonella* Enteritidis outbreaks attributable to PT4, on which the vaccines are based. Only 5 egg-associated outbreaks of PT4 infection were reported in England and Wales between 2007 and 2011.
- 1.13 Across the United Kingdom as a whole, there have also been similar changes in the epidemiology of human non-typhoidal salmonellosis over the last 4 decades. Between 1981 and 1991, the incidence of non-typhoidal salmonellosis in the United Kingdom rose by >170%, driven primarily by the epidemic of *Salmonella* Enteritidis PT4, which peaked in 1993 (O'Brien, S.J., 2013). Ref <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3563394/>
- 1.14 In 1981, *Salmonella* Enteritidis accounted for approximately 10% of human *Salmonella* illnesses in the UK, but by 1993 this proportion had risen to nearly 70%. In the early 1980s, PT4 overtook PT8 to become the predominant phage type in 1983, comprising 46% of isolations from human cases that year. By 1988 PT4 had risen to account for 81% of *Salmonella* Enteritidis isolates from human cases (O'Brien, S.J., 2013).
- 1.15 Lane *et. al.* (2014) analysed trends in reported incidents of *Salmonella* Enteritidis in chickens in Great Britain versus laboratory reporting of human *Salmonella* Enteritidis infections by this bacterium in England and Wales between 1985 and 2011; taking into account the impact of various interventions that have been introduced as time progressed (Figure 1). The authors of the paper stated that comparison of trends in reporting data, show that the rise in human *Salmonella* Enteritidis infections matched the rise in reported infections on chicken farms; layers, breeders and broilers are included in the data (although since the 2000s, most *Salmonella* Enteritidis incidents in poultry have been associated with laying hens, apart from one large outbreak of *Salmonella* Enteritidis PT21 in broilers in 2015).
- 1.16 Although reporting of incidents in chickens began to decrease in 1994 following introduction of voluntary national vaccination and flock hygiene programmes targeted at breeding flocks, this action had a limited effect on the trend in reported human infections (Lane *et. al.* 2014). Reports of outbreaks associated with *Salmonella* Enteritidis and chicken meat did, however, show a sharp decline from 1994 (Lane *et. al.* 2014). The reporting of egg-associated outbreaks did not start to decline until 1997, after the introduction of a *Salmonella* Enteritidis vaccination and the introduction of a flock hygiene

programme targeted at larger laying chicken flocks; this point marks a sharp decline in the human *Salmonella* Enteritidis epidemic. Lane *et al.*, (2014) concluded that the *Salmonella* Enteritidis epidemic was largely due to eggs because the earlier introduction of *Salmonella* controls in chicken meat production appeared to have a smaller impact on the course of the epidemic than that following the introduction of *Salmonella* controls in layers.

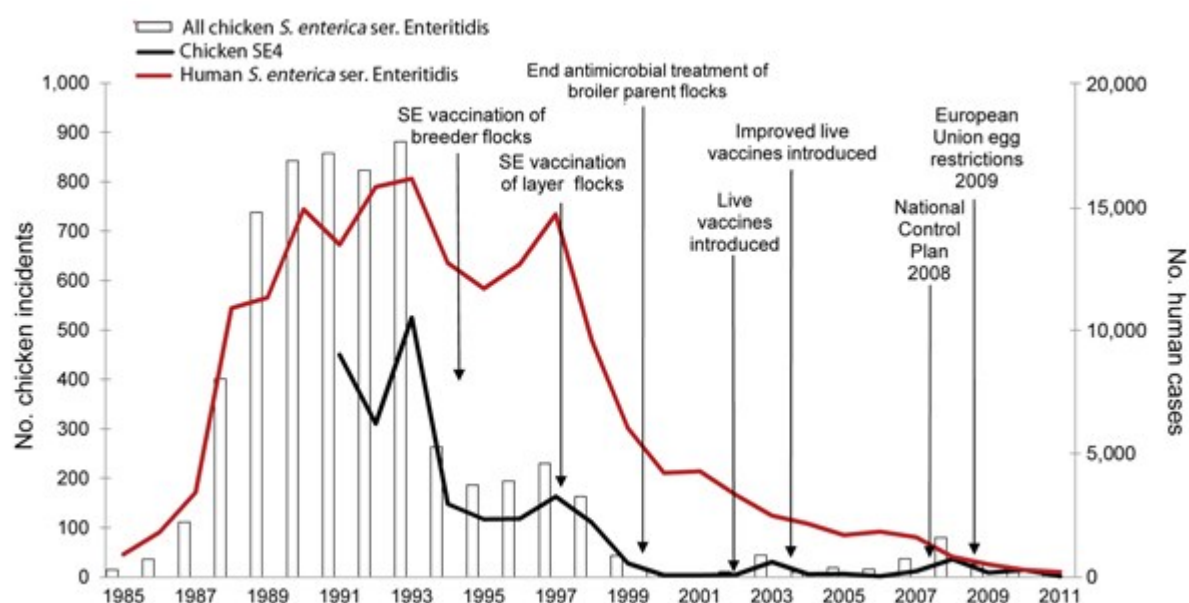


Figure 1 (Lane *et. al.*, 2014): Trends in the reporting of incidents of *Salmonella enterica* serovar Enteritidis in chickens in Great Britain versus laboratory reporting of human *S. enterica* serovar Enteritidis infections, England and Wales, 1985–2011.

- 1.17 Since the Committee's 2001 report, additional measures have been implemented nationally and/or EU-wide and are included in the above figure. In 2001, most use of attenuated vaccines was replaced by live ones, and in 2003, improved *Salmonella* Enteritidis auxotrophic live vaccines were adopted, and later used in conjunction with inactivated vaccines for additional protection (Lane *et. al.*, 2014). The National Control Programme (NCP) for *Salmonella* in commercial laying hen flocks was implemented in 2008 and set in place the monitoring and controls required in order to meet the legislative target for reduction in *Salmonella* prevalence (for the UK, a definitive target of a maximum of 2% of laying hen flocks to remain positive for *Salmonella* Enteritidis and/or *Salmonella* Typhimurium, including monophasic strains, per year; Regulation (EC) No. 517/2011). Both Government and industry share responsibility for implementation of the NCP (Defra, 2010). Additionally, the application of harmonised EU restrictions on the sale of fresh eggs from flocks

infected with *Salmonella* Enteritidis or *Salmonella* Typhimurium, began in 2009 and acted as a further incentive to improve farm standards, especially regarding rodent control (Carrique-Mas *et al.*, 2009, Davies and Carrique-Mas, 2010).

- 1.18 There are two data peculiarities in Figure 1 relating to two small increases in reported chicken *Salmonella* Enteritidis incidents in 2003 and 2008. The increase in 2003 occurred after early live vaccines were introduced and can be attributed to some farmers being unaware of the level of care needed to deliver the vaccine in the water supply properly; an education campaign was launched to address this (APHA personal communication). The 2007/8 increase can be attributed to additional intensive testing carried out by the egg industry to try and identify any residual infection before egg restrictions were introduced in 2009 (APHA personal communication).
- 1.19 Introduction of vaccination of laying hens in Scotland matched that in England – but the industry, and as such the *Salmonella* problem, was much smaller in Scotland. In N. Ireland, the rate of human infection with *Salmonella* Enteritidis increased in 1998 and again in 1999. At that time, vaccination of laying hen flocks against *Salmonella* Enteritidis was only just beginning and vaccine uptake was complicated by trade issues with the Irish Republic. However, the ACMSF in 2001 reported that more recent data subsequently showed a major drop in infection rate in N. Ireland.
- 1.20 Data from Scotland show that the rate of laboratory reporting since 1992 of human *Salmonella* Enteritidis is very similar to that in England and Wales. However, the trend in N. Ireland was very different. From 1992 to 1997, N. Ireland rates of laboratory reporting of *Salmonella* Enteritidis PT4 were much lower than in England, Wales and Scotland. After 1997, there was a rapid increase so that by 1999, the N. Ireland rate exceeded those in England, Wales and Scotland. However, in the first six months of 2000, there was a 43.1% fall in the number of cases of *Salmonella* Enteritidis in N. Ireland (ACMSF, 2001).
- 1.21 In the EU summary report on zoonoses, zoonotic agents and foodborne outbreaks 2014 (EFSA 2015), it was reported that, a total of 88,715 confirmed salmonellosis cases were reported by 28 EU Member States, resulting in an EU notification rate of 23.4 cases per 100,000 population. This represented a 15.3% increase in the EU notification rate compared with 2013. However overall there was still a statistically significant decreasing trend of salmonellosis in the 7-year period of 2008-2014. As in previous years, the two most commonly reported *Salmonella* serovars in 2014 were *Salmonella* Enteritidis and *Salmonella* Typhimurium, representing 44.4% and 17.4%, respectively, of

all reported serovars in confirmed human cases. The proportion of *Salmonella* Enteritidis increased compared with 2013. This increase was mainly attributed to an increase in cases in one Member State. In 2014, food-borne viruses were, for the first time, identified as the most commonly detected causative agent in the reported food-borne outbreaks (20.4% of all outbreaks), followed by *Salmonella* (20% of all outbreaks), although *Salmonella* was still the most frequent causative agent of strong-evidence outbreaks (38.2% of the outbreaks). The total number of *Salmonella* outbreaks within the EU decreased by 44.4% between 2008 (1,888 food-borne outbreaks) and 2014 (1,048 outbreaks). This corresponds with the decreasing trend in the number of human *Salmonella* cases in general. As in previous years, eggs and egg products were the most common identified food vehicles, associated with 44.0% of these outbreaks compared with 44.9 % in 2013.

- 1.22 Regulation (EC) No. 517/2011 implementing Regulation (EC) No 2160/2003 as regards a Union target for the reduction of the prevalence of certain *Salmonella* serotypes in laying hens of *Gallus gallus* and amending Regulation (EC) No 2160/2003 and Commission Regulation (EU) No 200/2010. The target specified is an annual % reduction from baseline or for a reduction to 2% or less flocks detected positive for *Salmonella* Enteritidis and *Salmonella* Typhimurium. The Regulation requires producers to monitor their flocks for *Salmonella* every 15 weeks during egg production and official sampling is required once annually in one flock on all premises with 1000 or more birds. The aim of the legislation is to optimise detection of infection, allowing the placing of egg marketing restrictions in the event a regulated serovar is detected.
- 1.23 Data obtained from hen flocks tested under the NCP in the UK between 2008 and 2014 (Figure 2, FSA, 2015) illustrate that there has been a decline in the proportion of laying flocks testing positive for regulated serovars (*Salmonella* Enteritidis, *Salmonella* Typhimurium and monophasic variants). The graph shows that the prevalence of regulated *Salmonella* serovars, as well as *Salmonella* species generally has been consistently below the legislative 2% positive target between 2008 and 2014 and significantly below the estimated 7.9% *Salmonella* Enteritidis and *Salmonella* Typhimurium UK prevalence in the 2004/2005 EU baseline survey (EFSA 2007).

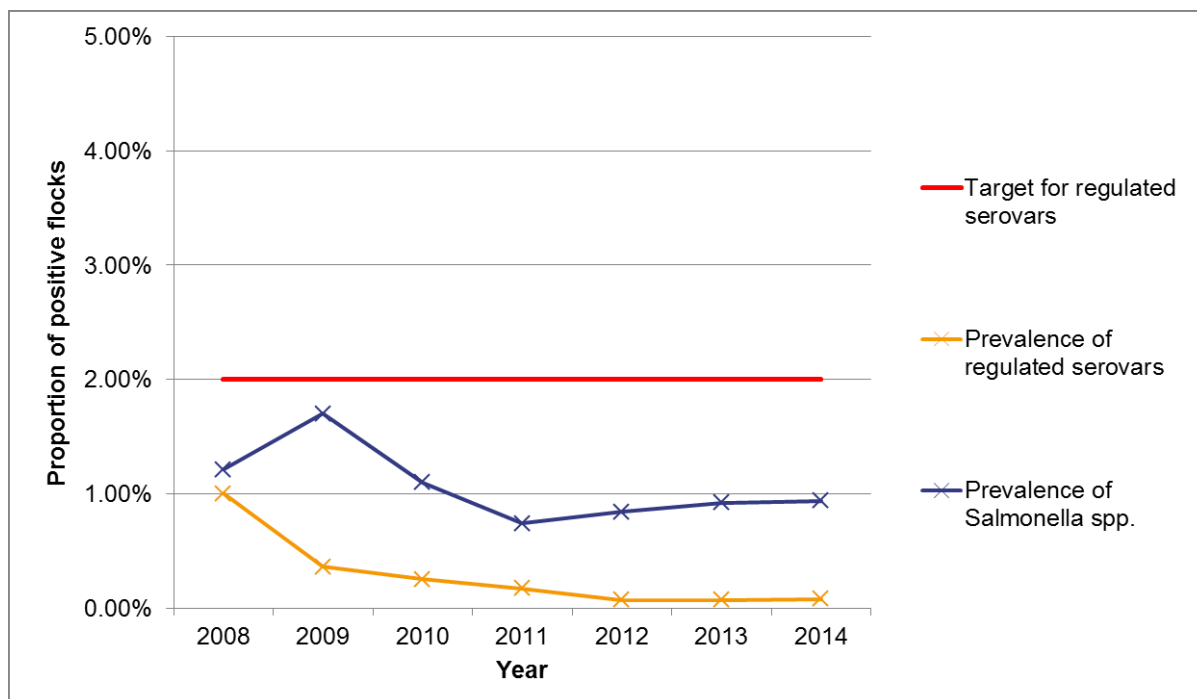


Figure 2: Prevalence of *Salmonella* spp. and the regulated serovars relative to the EU target in adult laying hen flocks of *Gallus gallus* in the UK *Salmonella* National Control Programme from 2008 – 2014

- 1.24 In Great Britain, confirmed infected flocks have to date all been voluntarily culled, following detection, as long term production of Class B eggs is not considered an economically viable option. This approach, although not a mandatory requirement of the legislation, has helped the UK achieve and maintain the lowest *Salmonella* Enteritidis national prevalence levels of the major poultry producing Member States (APHA personal communication).
- 1.25 Similarly, in the rest of Europe generally, a significant decreasing trend in prevalence of the target *Salmonella* serovars in all poultry populations in EU Member States has been reported in recent years. Regarding specifically the laying chicken sector, table 1 shows the reduction in prevalence seen in the six major egg producing countries since 2011. Overall, 23 EU Member States and three participating non Member States achieved the reduction target in 2014.

Country	Approximate number laying flocks 2014	Prevalence S. Enteritidis and S. Typhimurium 2014	Prevalence S. Enteritidis and S. Typhimurium 2013	Prevalence S. Enteritidis and S. Typhimurium 2012	Prevalence S. Enteritidis and S. Typhimurium 2011
Germany	5256	0.59%	1.18%	1.0%	1.2%
France	4928	1.16%	0.6%	1.4%	1.5%
Netherlands	3041	1.09%	0.72%	1.5%	2.2%
Poland	2362	1.91%	2.4%	2.8%	3.7%
Spain	2374	1.18%	1.87%	2.2%	2.8%
UK	3940	0.08%	0.07%	<0.1%	0.2%

Table 1: Prevalence of target *Salmonella* serovars in laying flocks detected during NCP sampling in the main egg producing MS 2011- 2014 (source EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks 2011 – 2014)

Changes from 2001 to present

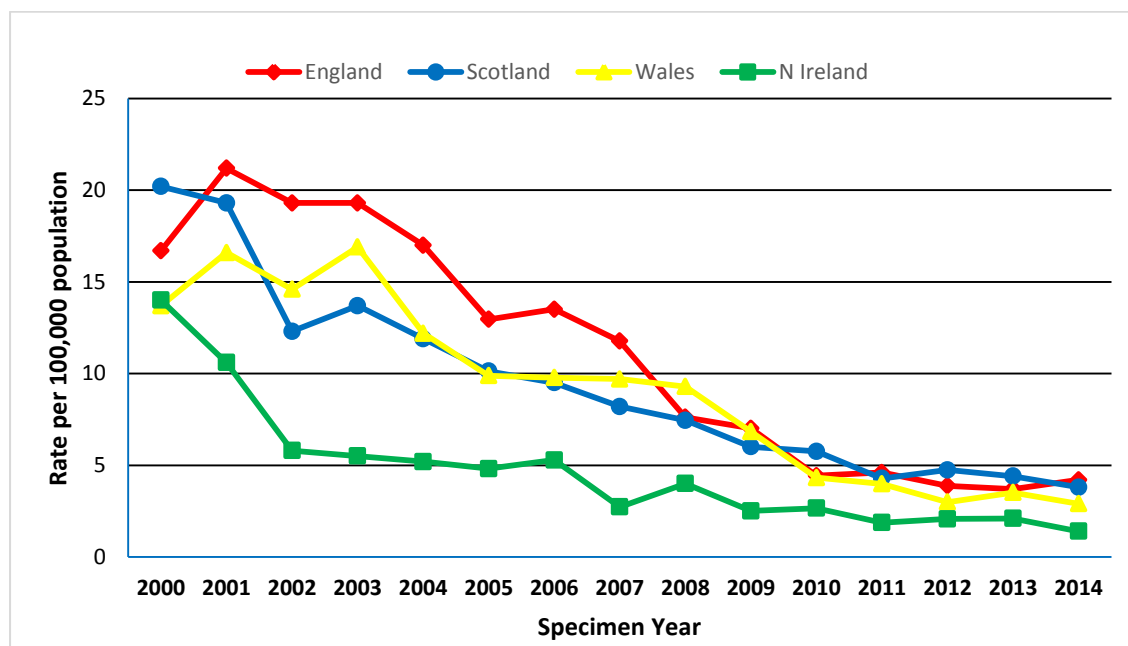


Figure 3. United Kingdom Incidence of *Salmonella* Enteritidis (all isolates), 2000 – 2014. (Epidemiology of Foodborne Infections Group 2015)

1.26 The decline in non-typhoidal *Salmonella* (NTS) infections has continued, with the numbers of cases and rates of infection remaining in decline in the UK since 2001 (Figure 3). The decline in *Salmonella* Enteritidis has continued in all

countries except England which saw an increase of 4% of reported NTS in 2014, reflecting the National outbreak of phage type 14b in summer (Table 2). Phage type 4 infections have continued to decline since 2001, primarily following interventions in the poultry and egg industries.

- 1.27 Between 2000 and 2014, *Salmonella* Enteritidis phage type 4 infections declined to a level equating to 2% of the figure seen in 2000. During this period of consistent decline, other phage types became noticeably more frequent in both reports and outbreaks (Figure 4). Most notable were phage types 14b (2004, 2009, 2011 and 2013); PT 8 (2007, 2012); PT 1 (2003 – 2006); and PT 6 (2001-2002, 2009). Many of these phage types were isolated in outbreaks associated with eggs, almost exclusively sourced from EU nations (Inns et al 2014, Janmohammed et al 2011, Vivancos et al 2013) esp. PT14b 2009, 2011, 2013).
- 1.28 During this same time frame, changes in the treatment of eggs in catering, and the increased use of pasteurised or heat treated liquid egg, along with changes in the UK population's food habits also assisted in reducing the numbers of outbreaks reported associated with eggs. During the 1990s outbreaks associated with consumption of eggs often involved dishes such as tiramisu and zabaglione, both involving raw eggs. These food types make rare appearances in the period between 2003 and 2014, with attribution more frequently associated with consumption of foods at oriental cuisine outlets (*Reference for PT 14b outbreaks especially*). Changes in production and flock management perceptively improved UK eggs and the number of cases and outbreaks of *Salmonella* Enteritidis and in particular, *Salmonella* Enteritidis PT4 declined. The removal of this backdrop of infection revealed the emergence of non PT4 as the predominant cause of outbreaks from 2002 onwards. The increased frequency of these outbreaks coincided with a dramatic increase in the number of eggs imported from Spain in 2001. Imported eggs from EU and non-EU countries are primarily destined for the catering market and independent shops that are not part of national retail chains. Problems with control in production in EU countries allowed for the introduction of potentially infected eggs into the UK catering market, which were first detected in 2002 with numerous investigations of egg outbreaks across the UK until 2004 when controls on the import of eggs from Spain were introduced (Gillespie and Elson 2005).

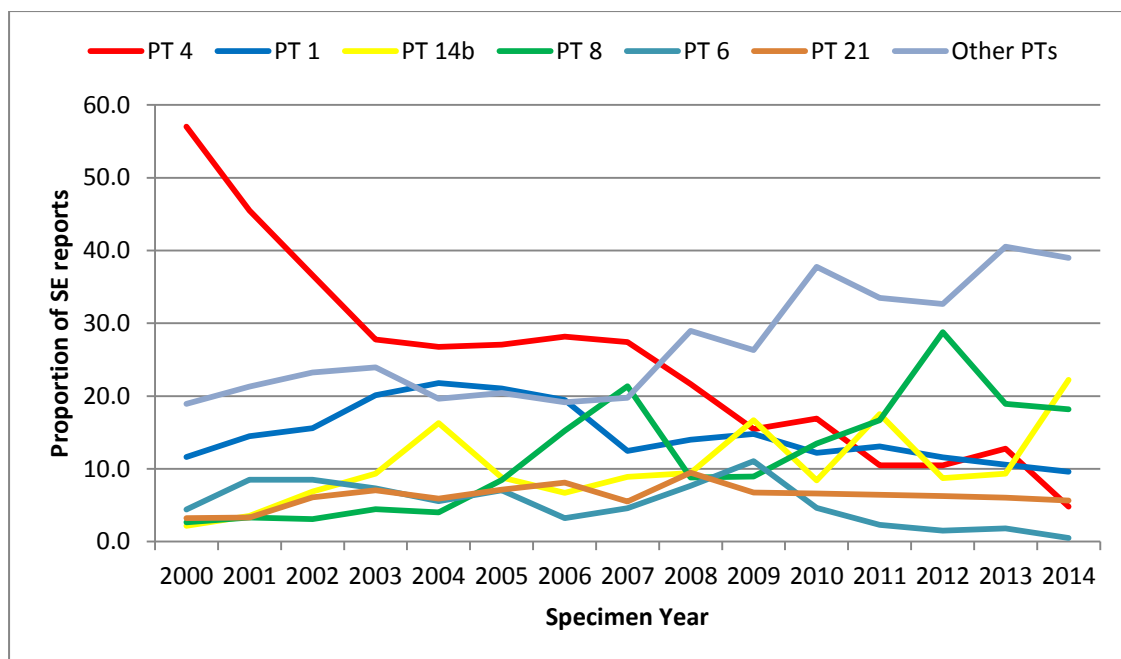


Figure 4 Proportionate reporting of predominant *Salmonella* Enteritidis phage types in England and Wales, 2000 – 2014

Non-UK eggs

- 1.29 Several studies have examined *Salmonella* contamination of non-UK eggs. The then Health Protection Agency examined outbreak-associated eggs during 2002 to 2004 and showed a higher rate of *Salmonella* contamination in or on eggs from outside the UK which are predominantly used in catering premises. Most *Salmonella* isolates were *Salmonella* Enteritidis non-PT4 (5.5% in Spanish eggs; 6.3% in eggs of country of origin not known). The study concluded that the use of Spanish eggs by the catering sector has been identified as a consistent significant factor in many of the outbreaks caused by *Salmonella* Enteritidis non-PT4 in England and Wales during 2002–2004. (HPA 2004, Little et al. 2007). <http://onlinelibrary.wiley.com/doi/10.1111/j.1472-765X.2007.02131.x/pdf>.
- 1.30 A number of different *Salmonella* Enteritidis PTs were reported in eggs of Spanish origin (PTs1, 1c, 3, 4, 5c, 6, 6a, 6d, 12, 13a, 14b, 35, 47, 58). *Salmonella* Infantis and *Salmonella* Livingstone were also reported to have been isolated from Spanish eggs. Similarly, contamination was more common for eggs of unknown origin and not Lion quality where PTs1, 2, 4, 6a, PT21 and 14b were reported. Additionally, *Salmonella* Altona, *Salmonella* Bredeney, *Salmonella* Infantis and *Salmonella* Ohio were also reported to be isolated from

such eggs. The study also found that 1.1% of UK non-Lion eggs were contaminated with *Salmonella* Enteritidis (PTs4, 6, and 24).

- 1.31 The FSA commissioned a survey of *Salmonella* contamination of non-UK eggs on retail sale in London and the North West of England over a period of 16 months, between March 2005 and July 2006. The estimated prevalence of all *Salmonella* and *Salmonella* Enteritidis was reported to be 3.3% and 2.6%, respectively (FSA, 2007).
- 1.32 Of the 157 *Salmonella* shell-positive samples, 10 were also contents positive (6 samples also contained two separate *Salmonella* isolates) making a total of 173 distinct *Salmonella* isolates recovered from the survey. From these, eight different serotypes were obtained, of which most were *Salmonella* Enteritidis (84.9%; 147/173). There were nine different PTs of *Salmonella* Enteritidis, with PT1 predominating (81.6%; 120/147). *Salmonella* Enteritidis PT4 was not detected. Other serotypes detected included *Salmonella* Mbandaka (14), *S.* Unnamed (untypable) (6), *Salmonella* Rissen (2), *Salmonella* Braenderup (1), *Salmonella* Infantis (1), *Salmonella* Panama (1) and *Salmonella* Weltevreden (1). The majority of the *Salmonella* isolates were resistant to one or more antimicrobial drugs (83.2%) of which most were resistant to nalidixic acid with reduced susceptibility to ciprofloxacin (78.6%). Nalidixic acid resistance is usually a marker for *Salmonella* of non-UK origin (Marimón et al., 2004).

Egg surveys

- 1.33 The FSA carried out a survey of *Salmonella* contamination of UK-produced shell eggs on retail sale in response to a recommendation by the Committee in its 2001 report (Food Standards Agency, 2004). The survey was carried out between March and July 2003. A total of 4753 samples (mostly boxes) of six eggs were purchased from a representative cross-section of retail outlets throughout the UK and the shell and contents tested for *Salmonella* contamination.
<http://tna.europarchive.org/20110116113217/http://www.food.gov.uk/science/surveillance/fsis2004branch/fsis5004eggs>
- 1.34 The overall finding was that nine samples (0.34%) were contaminated with *Salmonella*, which was equivalent to 1 in 290 “boxes” of 6 eggs. All *Salmonella* positive samples were from egg shells only. Comparison with the 1995/96 survey indicated that there had been a threefold reduction in the prevalence of *Salmonella* (from 0.99% to 0.34%). However, the most common *Salmonella* serovar isolated was still *Salmonella* Enteritidis.

- 1.35 There was no statistically significant difference between the prevalence of *Salmonella* contamination in samples purchased in England, Scotland, Wales or N. Ireland; or between the prevalence of *Salmonella* contamination in samples from different egg production types or between non-Lion code eggs and Lion code eggs or between eggs that were stored chilled or at ambient temperature, but the statistical power of the study was low because of the small number of isolates.
- 1.36 However, there was a statistically significant higher prevalence of *Salmonella* contamination of eggs from medium sized retailers than large ones.
- 1.37 Of the nine isolates from *Salmonella*-positive samples, seven (78%) were *Salmonella* Enteritidis and of these, three were *Salmonella* Enteritidis phage type 4 (PT4). There were also single isolates of *Salmonella* Infantis and *Salmonella* Livingstone. All of the *Salmonella* isolates were fully sensitive to 10 antimicrobial agents and none of the *Salmonella* Enteritidis PT4 isolates corresponded to known vaccine strains. *Salmonella* Infantis, *Salmonella* Livingstone and *Salmonella* Enteritidis PTs 4, 6 and 12 were found in previous egg surveys.
- 1.38 In addition to the nine *Salmonella* positive samples there were a further five egg samples from a limited time period within the laboratory which were reported as positive for *Salmonella* Dublin. This was an unusual and unexpected finding since this serovar is normally associated with cattle and on further investigation there appeared to be no evidence to support this finding in laying flocks. Whilst it is not possible to provide a definitive explanation for the *Salmonella* Dublin findings, it is most likely to have resulted from cross-contamination or sample identification error during the handling and testing of eggs in the laboratory, which was also testing meat samples. The Agency considered that there was sufficient doubt about the validity of the *Salmonella* Dublin findings to justify excluding them from the main analysis.
- 1.39 The interpretation of the main findings from the statistical analysis remains the same with or without the inclusion of the *Salmonella* Dublin findings.
- 1.40 The Agency carried out a survey of *Salmonella* contamination of raw shell eggs used in catering premises between November 2005 and January 2007. A total of 1,588 pooled samples of six eggs were collected at random from 1,567 catering premises in England, Wales, Scotland and Northern Ireland.
- 1.41 The overall finding was that six pooled samples were found to be contaminated with *Salmonella* on the shell of the egg giving a prevalence of 0.38%. Two

different serotypes were recovered of which the most common was *Salmonella* Enteritidis (5/6). There were three different PTs of this serovar with PT4 predominating (3/5). *Salmonella* Mbandaka, which is common but a relatively non-pathogenic serovar in animal feed and poultry flocks, was also isolated. *Salmonella* was detected from five egg samples comprising eggs that were UK produced and one from eggs produced in Germany. (The majority of eggs tested were UK eggs.) The survey's kitchen practice element showed evidence of poor egg storage and handling practices in catering premises (Food Standards Agency, 2007).

- 1.42 Between April and May 2003, a survey investigating the rate of *Salmonella* contamination in raw shell eggs from catering premises in the UK was carried out (Elson et al., 2005). A total of 34,116 eggs (5,686 pooled samples of six eggs) were collected from 2,104 catering premises, most of which were eggs produced in the United Kingdom (88%). *Salmonella* was isolated from 17 pools (0.3%) of eggs. Of these, 15 were *Salmonella* Enteritidis, which were further characterized to Phage types (PTs) as follows: PT6 (0.1%), PT4 (0.07%), PT12 (0.04%), PT1 (0.04%), and PT14b (0.02%). The authors reported that the *Salmonella* contamination rate of eggs produced in the UK appears to have decreased significantly since 1995 and 1996. This trend is reflected in the decrease of *Salmonella* Enteritidis and, in particular, PT4. *Salmonella* Livingstone and *Salmonella* Typhimurium definitive type 7 resistant to ampicillin, streptomycin, sulphonamides, and tetracycline were also isolated. <http://www.ncbi.nlm.nih.gov/pubmed/15726966>
- 1.43 A recent unpublished review of 36 egg surveys was carried out (Martelli et al. unpublished). The surveys were conducted in different years, using different bacteriological methods and for different purposes. The surveys were performed in a period ranging from 1991 to 2010 and in several countries: UK (14), Japan (4), USA (5), India (3), Ireland (3), Albania (1), Australia (1), Canada (1), France (1), Iran (1), New Zealand (1), and Uruguay (1). The results of the surveys can be summarised in Annex 2. The serovars isolated in the surveys are divided into *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* Other. Where available, a list of the *Salmonella* Other is provided in the table (Martelli et al., unpublished) at Annex II.
- 1.44 *Salmonella* Enteritidis is the serovar that was most prevalent in the majority of the surveys, but in some of the studies was not isolated at all, or was not the most prevalent serovar. (Martelli et al., unpublished).
- 1.45 In the past UK surveys, *Salmonella* Typhimurium appears to be isolated rarely from eggs when compared to *Salmonella* Enteritidis and to *Salmonella* Other. It

is possible to identify a peak of the presence of *Salmonella* Typhimurium in eggs during the early 1990s. It is possible to hypothesize that the peak observed in *Salmonella* Typhimurium egg contamination during the 1990s was due to a DT104 epidemic. In the surveys conducted after the 1990s, isolation of *Salmonella* Typhimurium from eggs in the UK has been very rare.

- 1.46 *Salmonella* Enteritidis also showed a peak during the early 1990s, and a progressive reduction over time. A higher *Salmonella* Enteritidis prevalence is reported in the study of Little et al. (2007) mentioned above, which was focused on non-UK eggs (from Belgium, France, Germany, Poland, Portugal, Republic of Ireland, Spain, The Netherlands) that were associated with a food-poisoning outbreak. The estimated prevalence of *Salmonella* Enteritidis-positive eggs was particularly high in this study and does not reflect the prevalence of *Salmonella* Enteritidis infection in UK eggs. Only 1.1% of the non-Lion code quality batches of eggs and 0% of the Lion code quality UK eggs tested positive for *Salmonella* in this study. None of the eggs originating from France, Germany, Portugal or the US were positive for *Salmonella*. A significant contamination rate (6.3%) was found in non- UK eggs of unknown provenance (Little et al., 2007).
- 1.47 *Salmonella* Other were reported in the UK studies (both from locally produced and from non-UK eggs). The most frequently isolated were *Salmonella* Infantis, *Salmonella* Livingstone, *Salmonella* Braenderup and *Salmonella* Virchow (Martelli et al., unpublished).

Duck eggs and other eggs

- 1.48 In July 2010, the then Health Protection Agency reported an unexpected increase in reports of *Salmonella* Typhimurium DT 8 infections in people in England and Northern Ireland (HPA, 2010b). By the end of October 2010, there were 81 laboratory confirmed human cases from all regions of England and Northern Ireland, an increase of 26% and 41% on the same period in 2009 and 2008, respectively. The descriptive epidemiological investigation found a strong association between infection and consumption of duck eggs. This was the first known outbreak of salmonellosis linked to duck eggs in the UK since 1949 and highlighted the continuing need to remind the public and commercial caterers of the potential high risks of contracting salmonellosis from raw or lightly cooked duck eggs.
- 1.49 In 2013 PHE carried out a study of duck eggs. Results from this study show that *Salmonella* was detected in two out of 145 six egg samples at a rate (around 1%) similar to that found in hen egg surveys carried out in the 1990s. The report makes reference to no outbreaks linked to duck eggs since 2012).

Foodborne outbreaks linked to eggs and egg products

- 1.54 The investigation and reporting of foodborne outbreaks has been mandatory for EU Member States since 2005 under Directive 2003/99/EC. The Directive makes provisions for such investigations and for close co-operation between authorities in the Member States. Thorough investigation of food-borne outbreaks aims to identify the pathogen, the food vehicle involved and the factors in the food preparation and handling contributing to the outbreak, providing information to facilitate risk management and improvement of food safety. Analysis of data from outbreak investigations for attributing human foodborne disease has been also described (Adak *et al* 2005), (Pires *et al* 2009).
- 1.55 A foodborne outbreak is defined by European legislation as ‘an incidence, observed under given circumstances, of two or more human cases of the same disease and/or infection, or a situation in which the observed number of human cases exceeds the expected number and where the cases are linked, or are probably linked, to the same food source’ (Directive 2003/99/EC). Starting in 2007, harmonised specifications on the reporting of food-borne outbreaks at the EU level have been applied. The specifications were revised in 2010 (EFSA 2011) with outbreaks now categorised as having ‘strong evidence’ or ‘weak evidence’ based on the strength of evidence implicating a suspected food vehicle.
- 1.56 Public Health England has operated a system of surveillance for general outbreaks of infectious intestinal disease (foodborne and non-foodborne) in England and Wales since 1992 (Cowden *et al*, 1995). Following the introduction of the statutory EU reporting requirements, to align with such requirements as well as modernising the system by enhancing and improving the capture of outbreak information, a stand-alone surveillance system, eFOSS (electronic Foodborne and non-foodborne gastrointestinal Outbreak Surveillance System), was implemented in 2009. The UK only collates and reports data for general outbreaks of foodborne infections². Data on household outbreaks (as opposed to outbreaks at private establishments involving members from more than one household) are not included in the dataset. Information including details of setting (in foodborne disease outbreaks defined as the place where food was prepared), mode of transmission, causative organism and details of epidemiological and laboratory investigations is collected. In addition, the type of evidence leading to the suspicion of a food vehicle is also collected and

² A general outbreak is an incident in which two or more people, from more than one household, or residents of an institution, thought to have a common exposure, experience a similar illness or proven infection (at least one of them having been ill).

therefore a distinction can be made between credibly identified vehicles, and vehicles assumed on the basis of, for example, biological plausibility (O'Brien *et al*, 2002). This system allows a more reliable evaluation of the contribution of different pathogens, foods, and settings than the biased sample represented by published investigations (O'Brien *et al*, 2006). Such data forms a national minimum dataset for analysis and reporting and the data are collated centrally by Gastrointestinal Diseases Department at the National Infection Service and reported annually to EFSA together with data from Scotland and Northern Ireland.

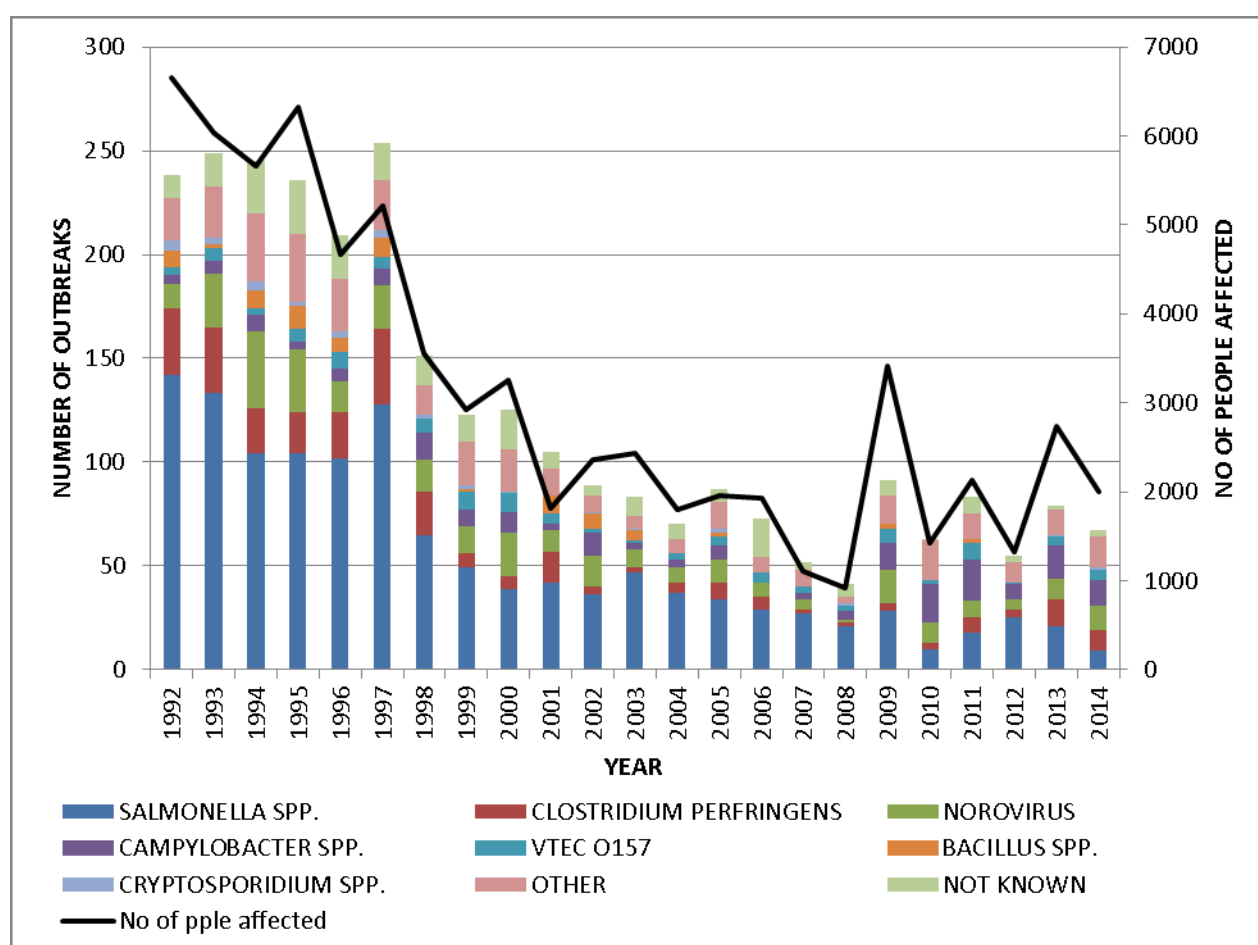


Figure 5 – general outbreaks of foodborne illness in humans, England and Wales 1992 - 2014

1.57 A recent review by Gormley *et al* reported that between 1992 and 2008, the number of foodborne outbreaks progressively declined from 238 in 1992 to 40 in 2008, with the exception of 1997 and 2005 (Gormley *et al*, 2011). The authors report that *Salmonella* species were implicated in almost half of all foodborne outbreaks but the proportion of *Salmonella* species outbreaks

collectively decreased significantly over the surveillance period. Specifically, the proportion of outbreaks caused by *Salmonella* Enteritidis PT 4 decreased significantly, and is attributed to the effect of successful intervention measures in UK poultry flocks. Outbreaks attributed to *Salmonella* Enteritidis non-PT 4 increased (mainly PT 1 and PT 14b) - with the greatest increases occurring from 2002, with a preponderance of these associated with eggs or egg dishes linked to food service establishments. It was noted that that these major resurgences were associated with substantive changes in market supply with the sourcing of eggs from other egg producers in Member States, where there is a lack of vaccination of layer flocks against *Salmonella* or controlled assurance. The authors also specifically noted the substantial rise in the number of outbreaks and sporadic cases of *Salmonella* Enteritidis PT 14b still occurring during the latter part of 2009 and associated with non-UK eggs linked to food service establishments.

- 1.58 A similar trend in the reduction of outbreaks caused by *Salmonella* has been seen at the EU level in recent years. In the 2014 annual report on European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks, EFSA reports that the total number of *Salmonella* outbreaks within the EU decreased by 44.4% between 2008 (1,888 food-borne outbreaks) and 2014 (1,048 outbreaks). This corresponds with the decreasing trend in the number of human *Salmonella* cases in general. Although the proportion decreased from 59.9% in 2013 to 46.1% in 2014, as in previous years, 'eggs and egg products' were still the most frequently identified food vehicles, associated with 44.0% of the reported *Salmonella* strong-evidence outbreaks (44.9% in 2013). France, Poland and Spain together reported 69.7% of these outbreaks. For those food-borne outbreaks where a phage type (PT) was reported, the most common type reported was S. Enteritidis PT8 and PT14b. (EFSA, 2015).

General outbreaks of foodborne disease in England and Wales 2009 – 2014 linked to eggs and/or egg products

- 1.59 Table 2 shows the 26 reported outbreaks of general foodborne disease with confirmed or putative links to eggs and / or egg products, reported in eFOSS for the years 2009 – 2014. The data presented is based on the data reported in eFOSS, the individual outbreak reports and published information.
- 1.60 The largest outbreaks were reported in 2009, 2011 and 2014, in all three cases caused by *Salmonella* Enteritidis PT 14b, with a confirmed link to eggs imported from the EU determined through microbiological and analytical epidemiological evidence.

Table 2 – Foodborne outbreaks reported on eFOSS, in publications and in outbreak reports from 2009 to 2014 where a confirmed or putative link to chicken eggs (table eggs and / or egg products) has been reported.*

Year	PHE Region	Organism identified	No. of lab cases	Hospitalisation	No. of deaths	Setting	Food vehicle (s)	Origin of food vehicle	Evidence**	Notes***
2009	MIDLANDS & EAST OF ENGLAND	SALMONELLA ENTERITIDIS PT1	8	0	0	Restaurant	Eggs	Not known	Descriptive epidemiological	
2009	WALES	SALMONELLA ENTERITIDIS PT 14B	5	1	0	Restaurant	Rice - egg fried and rice - chicken fried	Not known	Descriptive epidemiological	NxCpl ¹ Suspect imported eggs
2009	SOUTH OF ENGLAND	SALMONELLA ENTERITIDIS PT 14B	3	0	0	Chinese take-away	Eggs	Not known	Microbiological	NxCpl ¹ Suspect imported eggs
2009	NORTH OF ENGLAND	SALMONELLA ENTERITIDIS PT 5A	5	0	0	Restaurant	Mayonnaise - garlic mayonnaise	Not known	Microbiological and Descriptive epidemiological	
2009	LONDON	SALMONELLA ENTERITIDIS PT 14B	3	0	0	Café	Mayonnaise - made with raw shell eggs	Not known	Microbiological	NxCpl ¹ . Suspect imported eggs
2009	NORTH OF ENGLAND	SALMONELLA ENTERITIDIS PT 14B	2	0	0	Mobile caterer	Eggs - consumed raw	Not known	Descriptive epidemiological	NxCpl ¹ . Suspect imported eggs
2009	NATIONAL	SALMONELLA ENTERITIDIS PT 14B	489	27	2	Restaurant	Eggs - various egg dishes and deserts	Intra-EU trade	Microbiological, Descriptive epidemiological and Analytical epidemiological evidence (cohort study)	NxCpl ¹
2009	LONDON	SALMONELLA ENTERITIDIS PT 4	33	2	0	Prison	Egg and cress sandwiches	Domestic	Descriptive and analytical epidemiological (retrospective cohort study)	S ²

2010	SOUTH OF ENGLAND	SALMONELLA ENTERITIDIS PT6A	7	0	0	Chinese take-away	Rice - egg fried	Not known	Microbiological and Descriptive epidemiological	
2010	NATIONAL	SALMONELLA TYPHIMURIUM	81	5	1	Convenience mini-market	Duck eggs	Domestic	Microbiological and Descriptive epidemiological	
2011	MIDLANDS & EAST OF ENGLAND	SALMONELLA ENTERITIDIS PT6	14	0	0	Bakers	Mayonnaise - made with rse	Domestic	Microbiological and Descriptive epidemiological	
2011	MIDLANDS & EAST OF ENGLAND	SALMONELLA ENTERITIDIS PT 4	2	0	0	Nurse care home	Egg dish - unspecified	Domestic	Descriptive epidemiological	
2011	NORTH OF ENGLAND	SALMONELLA ENTERITIDIS PT14b	23	0	0	Restaurant	Rice - egg fried rice and special fried with chopped ham	Intra-eu trade	Microbiological and Descriptive epidemiological	NxCpl ¹ . Strong evidence
2011	NORTH OF ENGLAND	SALMONELLA ENTERITIDIS PT14b	6	0	0	Restaurant	Rice - egg fried	Intra-EU trade	Analytical epidemiological evidence (cohort study)	NxCpl ¹ . Strong evidence
2011	MIDLANDS AND EAST OF ENGLAND	SALMONELLA ENTERITIDIS PT14b	10	0	0	Restaurant	Egg dish - unspecified	Intra-EU trade	Microbiological and Descriptive epidemiological	NxCpl ¹ . Strong evidence
2011	NATIONAL	SALMONELLA ENTERITIDIS PT14B	263	39	1	Restaurant	Egg dish - unspecified	Intra-EU trade	Microbiological and Descriptive epidemiological	NxCpl ¹ . Strong evidence
2011	LONDON	SALMONELLA ENTERITIDIS PT3	6	0	0	Fast food establishment	Not known	Not known	None	Weak evidence
2012	NORTH OF ENGLAND	SALMONELLA ENTERITIDIS PT4	8	1	0	Restaurant	Dish made with eggs - yorkshire pudding and mixed carvery meats	Domestic	Descriptive epidemiological	Weak evidence

2012	NORTH OF ENGLAND	SALMONELLA ENTERITIDIS NON-PT4	4	0	Unknown	Chinese take-away	Dish made with eggs	Not known	Descriptive epidemiological	Weak evidence
2012	NATIONAL	SALMONELLA ENTERITIDIS PT 1	9	2	0	Health food shops	Pasteurised liquid egg white	Not known	Microbiological and Descriptive epidemiological	NxCpl ¹ . Strong evidence
2013	MIDLANDS AND EAST OF ENGLAND	SALMONELLA ENTERITIDIS PT 56	7	2	0	Restaurant	Chicken and rice - egg fried	Not known	Analytical epidemiological evidence (cohort study)	Strong evidence
2014	MIDLANDS AND EAST OF ENGLAND	SALMONELLA ENTERITIDIS PT 14B	15	5	0	Restaurant	Egg noodles	Intra-EU trade	Microbiological, Descriptive epidemiological and WGS	Strong evidence
2014	NORTH OF ENGLAND	SALMONELLA ENTERITIDIS PT 14B	32	3	0	Chinese take-away	Eggs	Intra-EU trade	Microbiological, Descriptive epidemiological and WGS	Strong evidence
2014	MIDLANDS AND EAST OF ENGLAND	SALMONELLA ENTERITIDIS PT 14B	32	3	3	Hospital	Chicken dishes and eggs	Intra-EU trade	Descriptive epidemiological and WGS	Strong evidence
2014	SOUTH OF ENGLAND	SALMONELLA ENTERITIDIS PT14B	100	3	0	Restaurant	Eggs	Intra-EU trade	Microbiological, Descriptive epidemiological and Analytical epidemiological evidence (case control study) and WGS	Strong evidence
2014	LONDON	SALMONELLA ENTERITIDIS PT14B	9	5	0	Kebab shop	Eggs	Not known	None	Weak evidence. Suspect imported eggs

¹ Nalidixic acid and low level ciprofloxacin resistance

² Sulphonamide resistance

*Data extracted from eFOSS 14/01/2015

**Type of evidence:

- Descriptive epidemiological evidence: suspicion of a food vehicle in an outbreak based on the identification of common food exposures, from the systematic evaluation of cases and their characteristics and food histories over the likely incubation period by standardised means (such as standard questionnaires) from all, or an appropriate subset of, cases.
- Microbiological evidence: detection of a causative agent in a food vehicle or its component or in the food chain or its environment combined with detection in human cases, or clinical symptoms and an onset of illness in outbreak cases compatible with / pathognomonic to the causative agent identified in the food vehicle or its component or in the food chain or its environment.
- Analytical epidemiological evidence: a statistically significant association between consumption of a food vehicle and being a case in an outbreak demonstrated by studies such as a cohort study, a case-control study or similar studies

*** Strength of evidence:

Categorisation according to EFSA technical specifications is included from 2011. Strong epidemiological evidence includes statistical associations in well-conducted analytical epidemiological studies or convincing descriptive evidence. Product-tracing includes investigating the movement of a food product and its constituents through the stages of production, processing, and distribution. Microbiological evidence includes the detection of the causative agent in the food vehicle or its component, and the detection of the causative agent in the food chain or from the preparation or processing environment. Microbiological evidence has always to be combined with detection of the causative agent from the human cases or symptoms in the human cases that are pathognomonic to the causative agent. Descriptive environmental evidence alone is almost invariably weak (EFSA, 2015).

- 1.61 There were 16 recognised, discrete local outbreaks of *Salmonella* Enteritidis PT 14b with resistance to nalidixic acid and low susceptibility to ciprofloxacin (NxCpl) in England and Wales between August and December 2009. All but one of these outbreaks were linked to food-service premises; the remaining outbreak was linked to a residential care home for the elderly. The total number of reported cases associated with these outbreaks was 152: six were hospitalised and two deaths were reported. A national case control study found associations with food consumption at restaurants serving Chinese or Thai cuisine, egg consumed away from home, and eating vegetarian foods away from home. The outbreak strain, indistinguishable by molecular diagnostic testing from isolates obtained from human cases of *Salmonella* Enteritidis PT14 NxCpL infection, was isolated from samples of eggs sourced from a single production establishment in Spain, as well as in egg products (egg mayonnaise, egg-fried rice, pooled liquid egg mix) and on work surfaces in food premises, indicating the contributory role of cross contamination (Janmohamed *et al*, 2011).
- 1.62 Again in 2011, there were several recognised, discrete local outbreaks of *Salmonella* Enteritidis PT 14b NxCpl. The largest reported national outbreak involved a total of 263 laboratory confirmed cases of non-travel related *Salmonella* Enteritidis PT14b in England & Wales, with 39 hospitalisations and one reported death. At least six restaurants were linked to outbreaks of two or more confirmed cases. Strong microbiological and descriptive epidemiological evidence indicated that the outbreak was caused by consumption of foods contaminated (or cross-contaminated) with an indistinguishable strain of *Salmonella* from eggs from a single source, which on the basis of the supply tracing investigation (mapping of the egg supply network) was most likely to be a single chicken flock on the premises of a major egg producer in Spain. Microbiological typing indicated that the *Salmonella* Enteritidis PT 14b NxCpl isolates from egg samples were indistinguishable from the majority of the human isolates as well as an indistinguishable profile to that identified in the 2009 outbreak. *Salmonella* Enteritidis was detected by the Spanish authorities in faecal samples taken at one of the buildings at the implicated Spanish producer and following this all birds in the affected building were compulsorily slaughtered.
- 1.63 In 2014, several outbreaks of *Salmonella* Enteritidis PT14b were investigated in England and Wales. In total, 287 cases met the outbreak case definition and a national outbreak investigation was instigated. Seventy-eight (27%) cases were reported to have been hospitalised (of whom 61 were not thought to have acquired their infection while in hospital). During the same period, outbreaks caused by a *Salmonella* Enteritidis strain with the same specific multilocus variable-number tandem repeat analysis (MLVA) profile shared by some of the

domestic cases occurred in other European Union Member States. Food trace-back investigations in the UK and other affected European countries linked the outbreaks to chicken eggs from a German company. Whole genome sequencing results showed that 332 clinical and environmental samples from Austria, France, Germany, Luxembourg and the UK and eight isolates from the implicated egg production company in Germany were all closely genetically related. During this investigation, no eggs supplied by the German producer were found in the UK for testing and the investigators considered that this most likely reflected the delay between egg consumption, symptom onset, phage typing, food history taking and egg sampling (Inns *et al*, 2015).

- 1.64 Five of the outbreaks reported to eFOSS between 2009 and 2014 were linked or had putative links to UK sourced eggs. One of these outbreaks, the *Salmonella* Typhimurium DT8 outbreak in 2010 was associated with the consumption of duck eggs (Noble *et al*, 2011). The remainder of the outbreaks, which resulted in a reported 57 laboratory confirmed cases, 3 hospitalisations but no deaths, were linked to UK produced chicken eggs.
- 1.65 In the investigation into the *Salmonella* Enteritidis PT4 outbreak in a prison in 2009, the analytical epidemiological investigation demonstrated a strong association between eating egg cress rolls and illness, pointing strongly to the eggs being the source of this outbreak. The Environmental Health investigations identified several deficiencies in the egg pooling, cooking and storage methods used in the kitchen as possible contributory factors. The eggs used by the prison kitchen were all supplied by a single large catering supplier and were Lion branded. None of the eggs used in the egg cress roll preparation were available to sample. However, 120 eggs which had the same “best before date” as those used in the egg cress sandwiches were tested with negative results. Other food and environmental samples did not yield any positive *Salmonella* results (Davies *et al*, 2013).
- 1.66 A *Salmonella* Enteritidis PT6 outbreak in 2010 was reported at a bakery with putative links to mayonnaise made with raw shell eggs used in sandwiches. The outbreak strain was not isolated from eggs but was isolated from environmental swabs, including a dishcloth. The source was believed to be old improperly cleaned plastic tubs used by staff to mix egg mayonnaise. The eggs used at the bakery were sourced from a UK supplier who was not a member of the Lion Scheme. In an outbreak of *Salmonella* Enteritidis PT 4 at a golf club restaurant in 2012, several food vehicles, including Yorkshire pudding that was made with pooled raw shell eggs, were identified as possible vehicles of transmission but food and environmental samples did not yield any positive *Salmonella* results. The eggs used at the restaurant were UK stamped but not Lion branded eggs. Overall, only weak descriptive epidemiological evidence

pointed to eggs being a possible source of the outbreak. Similarly, descriptive epidemiological evidence from an investigation of a *Salmonella* Enteritidis PT 4 outbreak in a residential care home in 2011 indicated UK sourced eggs used in dishes at the care home as being the likely food vehicle in the outbreak. *Salmonella* was not isolated from any food samples or environmental swabs and the evidence for link to the Yorkshire pudding was classified as weak evidence so only a putative link could be established. The egg supply was from a UK producer who was not a member of the Lion Code Scheme and the eggs had been supplied through an unregulated egg supplier whose licence had been revoked.

- 1.67 In total, 12 of the reported 26 outbreaks reported in eFOSS since 2009 do not include a report of the origin of the implicated or suspected eggs or egg products. In some cases, this is due to the weak evidence linking eggs as a probable vehicle of infection. In other cases, even though eggs are linked by strong analytical epidemiological evidence as a vehicle of infection and a suspect or likely origin could possibly be inferred from the descriptive epidemiology, published literature or from the specific serovar/phagetype/antimicrobial resistance pattern for the isolates obtained from the human diseases cases, in the absence of reliable and complete egg tracing information, a confirmed origin for the eggs cannot be reported.
- 1.68 In some of the reported outbreaks, where the only evidence is descriptive epidemiological evidence implicating eggs or egg products, this evidence is generally categorised as weak evidence based on the EFSA specifications. For example, for the reported *Salmonella* Enteritidis PT3 outbreak in 2011, the outbreak investigation indicated weak evidence from an analytical study comparing cases with historical controls to suggest that cases were significantly more likely to eat at Chinese restaurants than controls, however no specific epidemiological or food exposure links were found for the majority of cases. Two cases were linked to particular food outlets resulting in an investigation being carried out in nine restaurants by Environmental Health Officers (EHOs), but no common poultry suppliers or common egg batches were identified and food and environmental sampling yielded no significant findings. Without, further microbiological or analytical epidemiological evidence to back up the putative link, eggs and/ or egg products can only be linked as possible food vehicles.
- 1.69 Even the availability of analytical epidemiological evidence does not always allow for the conclusive identification of a food vehicle. For example, in the *Salmonella* Enteritidis PT 56 outbreak in 2013, linked to various food establishments' particularly oriental restaurants, the univariate analysis of the outbreak analytical study identified an association with consumption of egg fried

rice. The pre-planned analysis of all rice dishes was undertaken (rice dishes were combined due to multiple use of ladles in all rice dishes), and identified a strong association with consumption of a rice dish. Tracing of supply chains focused on eggs and chicken, as existing literature on previous outbreaks had implicated these food types and the outbreak analytical investigation had implicated egg containing dishes. As no specific egg batch numbers were available due to the time lapse between exposure and cases being confirmed, the investigators reported that it was impossible to trace the origin of specific eggs. The investigators noted that records are not routinely kept of egg batch numbers by suppliers, only commercial records of quantity, therefore such tracing could only identify if a company was part of a supply network, not whether the recipient at the end of the supply chain had received eggs from a specific supplier if it was one or more steps removed. During this outbreak investigation, particular efforts were made to identify the entire UK supply chain for the implicated restaurants but this also failed to identify a common supplier/source. Due to the fluctuating nature of the egg market, price variations and the large volume of eggs being handled by large suppliers, suppliers are regularly changed. Food outlets may use eggs from different sources on a daily basis. As also noted by the authors of the publication on the outbreak of *Salmonella* Enteritidis PT14b linked to a German egg producer (Inns *et al*, 2015) as the national investigations into general foodborne outbreaks generally begin sometime after cases reported consuming food from a particular implicated food outlet, eggs present on the site at the time of the investigation are not necessarily from the same supplier or producer, making tracing of potentially contaminated eggs difficult. Improved tracing information could however result in provisional identification of egg producers that could be reasonably linked to a specific outbreak, leading to further investigations and sampling on suspect farms, increasing the likelihood of identifying a specific infected poultry flock so that risk management measures can be taken.

Foodborne disease outbreaks in England and Wales linked to egg products

- 1.70 Since 2000, there has also been an increase in the use of heat treated/pasteurised liquid egg in both the catering industry and latterly in the health foods industry. In 2007 there was a significant outbreak of *Salmonella* Enteritidis PT 1e in England, Scotland, Wales and Jersey. Foods associated in different clusters of the outbreak all involved the use of pasteurised egg from a single producer based in France. In many of the clusters investigated, it was noted that caterers specifically used pasteurised egg produce following standard advice from the Food Standards Agency. The pathogen was isolated from both liquid egg white and liquid egg yolks in packages of 1 litre, and was indistinguishable from isolates in humans when compared by PFGE.

- 1.71 In 2012, an outbreak was reported in England associated with a liquid egg product marketed through the internet, primarily aimed at body builders. *Salmonella* Enteritidis PT 1 was isolated from patients in England and Scotland, and also isolated in the raw product in Austria.
- 1.72 A third event involving pasteurised egg was reported in 2014, following a birthday party in North London after which a number of children were reported ill and confirmed with *Salmonella* Enteritidis PT 21. The bakery that provided the cake for this and 2 other similar events used liquid pasteurised eggs to make the cake filling, on the basis that the product should be free from *Salmonella*. Samples of the liquid egg product were tested and found negative.
- 1.73 These three events highlight that pasteurised egg products, whilst at the time being considered to pose less risk than raw egg, still carry a small risk of contamination. Given the increasing pressure placed by authorities on caterers who produce under cooked egg products to use pasteurised egg products, the potential for these products to become a problem in the future needs to be assessed. In all three events, the product originated from the same producer in the EU, potentially indicating failures in their processing or procedures. In the mainland UK, eggs from positive flocks are not diverted to processing into egg products, whereas in many other EU countries this is normal practice, therefore the risk from domestically produced egg products will be lower.

Conclusions

- 1.74 *Salmonella* Enteritidis has been responsible for occasional cases of infection in the UK and elsewhere for over 100 years. However, its emergence as a pandemic chicken meat- and egg-associated global public health problem in the late 1980s caused the largest and most persistent epidemic of foodborne infection attributable to a single subtype of any pathogen.
- 1.75 In England and Wales, it is estimated that >525,000 people became ill during the course of the epidemic. The epidemic was associated with the consumption of contaminated chicken meat but more importantly, shell eggs. A decline in numbers of infections started after the introduction of vaccination for *Salmonella* Enteritidis and other control measures in chicken breeding and the production and distribution of eggs and chicken meat.
- 1.76 Data obtained from hen flocks tested under the National Control Programme in the UK between 2008 and 2014 illustrate that there has been a decline in the proportion of laying flocks testing positive for regulated serovars (*Salmonella* Enteritidis, *Salmonella* Typhimurium and monophasic variants).

- 1.77 In the UK, the prevalence of regulated *Salmonella* serovars, as well as others has been consistently below the legislative 2% positive target between 2008 and 2014 and significantly below the estimated 7.9% *Salmonella* Enteritidis and *Salmonella* Typhimurium UK prevalence in the 2004/2005 EU baseline survey.
- 1.78 In Great Britain, confirmed infected flocks have to date all been voluntarily culled, following detection of *Salmonella*. This approach, although not a mandatory requirement of the legislation, has helped the UK achieve and maintain the lowest *Salmonella* Enteritidis national prevalence levels of the major poultry producing Member States.
- 1.79 Since 2009, only 4 small general outbreaks have been linked to UK produced eggs and only one outbreak in 2009 linked to eggs produced by a member of a certified farm assurance scheme. The evidence implicating contaminated eggs as the vehicle of transmission was analytical epidemiological information only as no egg samples tested at the time yielded positive *Salmonella* results. Of the 26 general foodborne outbreaks linked to eggs or egg products reported to eFOSS since 2009, nine outbreaks have been definitively linked to imported eggs. Outbreaks of *Salmonella* Enteritidis PT14b in 2009, 2011 and 2014 linked to imported eggs were especially large outbreaks, with a reported 852 confirmed laboratory cases of human disease in total. A further five outbreaks reported since 2009 had suspected links to imported eggs.
- 1.80 Several outbreak investigations have not been able to determine a specific food source and the difficulties in obtaining food already eaten as well as linking food sources through complex supply chains limits effective investigation. Tracing of suspect batches of eggs is complicated by the fact that specific egg batch numbers are usually not available due to the time lapse between exposure and outbreak investigation making tracing of potentially contaminated eggs difficult.
- 1.81 Recent outbreaks of salmonellosis linked to imported pasteurised egg products highlights that, whilst at the time being considered to pose less risk than raw egg, these products still carry a small risk of contamination and the potential for these products to become a problem in the future needs to be assessed.
- 1.82 Advice concerning the risk associated with eating raw and lightly cooked eggs was developed when reported human *Salmonella* Enteritidis infections were markedly higher than they are now. Although human cases linked to eggs continue to occur these are much less frequent than in the 1990s and there have been no outbreaks linked to Lion branded eggs in the last nearly 7 years.

- 1.83 Between 2000 and 2014, changes in the treatment of eggs in catering, and the increased use of pasteurised or heat treated liquid egg, along with changes in the UK population's food habits also assisted in reducing the numbers of outbreaks reported associated with eggs.
- 1.84 In the past UK egg surveys, *Salmonella* Typhimurium appears to be isolated rarely from eggs when compared to *Salmonella* Enteritidis and to *Salmonella* Other (serovars other than Typhimurium and Enteritidis).
- 1.85 In July 2010, the then Health Protection Agency reported an unexpected increase in reports of *Salmonella* Typhimurium DT 8 infections in people in England and Northern Ireland. This was the first known outbreak of salmonellosis linked to duck eggs in the UK since 1949 and highlighted the continuing need to remind the public and commercial caterers of the potential high risks of contracting salmonellosis from raw or lightly cooked duck eggs.
- 1.86 In 2013 PHE carried out a study of duck eggs. Results from this study show that *Salmonella* was detected in two out of 145 six egg samples at a rate (around 1%) similar to that found in hen egg surveys carried out in the 1990s.

Recommendations

- 1.87 We recommend that the Agency and other Government Departments continue to monitor UK egg outbreaks associated with *Salmonella* Enteritidis and other microbiological hazards and ensure that the Committee is updated regularly to ensure that our advice is reviewed and updated as necessary.
- 1.88 We acknowledge the significant efforts undertaken by the UK egg industry since the 1990s to reduce the prevalence of *Salmonella* Enteritidis in laying hen flocks which in turn has made a remarkable impact in reducing the level of human *Salmonella* Enteritidis infections in humans.
- 1.89 We recommend that at timely intervals and with resources permitting, that regular surveys are carried out to assess the level of *Salmonella* contamination of hens' eggs on retail sale and used in catering establishments in the UK and that the origins of any contaminated eggs are recorded. We would like to be kept informed of the outcomes of any surveys.
- 1.90 We recommend that further data are gathered on sales of other types of eggs including duck and quails eggs and if possible we recommend that further data are gathered at regular intervals to assess the contamination levels of such eggs.

1.91 We recommend that measures to improve the traceability of egg supplies, especially those within the catering sector, be considered.

Chapter 2: Identification of microbiological hazards associated with eggs and egg products

Overview

- 2.1 A wide range of pathogens of potential public and animal health risk can be found in laying hens and other table egg-producing birds. A small number of these are considered to be truly vertically transmitted, and so can be found within the confines of the shell, having been deposited within the forming egg, including its membranes, during the development of the oocyte in the ovary or its transmission down the reproductive tract.

Viruses

- 2.2 Below is a list of potentially vertically transmitted zoonotic viruses:
- *Leucosis/sarcoma group of avian type C viruses*
 - *Reticuloendotheliosis viruses of REV group*
 - *Tremorvirus*
 - *Avian encephalomyelitis virus*
 - *Avian adenovirus*
 - *Avian influenza virus*
- 2.3 Although examples of human infection with these viruses exist, there is no evidence that handling, or consumption of table eggs is involved and infections are likely to be acquired through direct contact with infected poultry, usually via the respiratory route in a confined, dusty environment, or in situations in which living accommodation is shared with infected poultry. Only avian influenza virus is associated with a significant number of human cases, but is very rare in the UK.
- 2.4 These viruses may also be present on the surface of eggs due to their occurrence in faeces of laying birds, but their persistence characteristics in this situation are not known.

Bacteria

- 2.5 *Salmonella* is the most commonly recognised bacterial species that can be vertically transmitted. *Salmonella* Gallinarum and its biovariant *Salmonella* Pullorum are host-adopted to avians and zoonotic infection is not expected, and is extremely rarely reported. The turkey-pathogenic group K. *arizonae* serovar 018:z4,z23, is similarly transmitted and rarely reported from humans. All of these serovars are not considered to be present in commercial scale poultry in GB, but *Salmonella* Pullorum may occasionally be found in backyard chickens

or game birds, including commercially reared birds for shooting. *Salmonella* Enteritidis, which is closely related to *Salmonella* Gallinarum, can also be vertically transmitted, but the efficiency of this varies with genovar, and will be discussed in more detail later. Other serovars such as *Salmonella* Heidelberg, *Salmonella* Infantis, and *Salmonella* Typhimurium may also be vertically transmitted to some extent, but this is very variable according to strain and the management of laying flocks. In recent years monophasic strains of *Salmonella* Typhimurium have occasionally been reported from flocks of laying hens, and although these have been increasing in humans since 2007, mainly associated with infection originating from the porcine reservoir, eggs have not been associated with infection and infection in laying flocks appears to be relatively transient .

Horizontal transmission leading to internal contamination of eggs

2.6 It is possible for micro-organisms to enter intact eggs if cooling takes place in a moist environment such that a negative pressure gradient is created that draws fluids and suspended micro-organisms into the egg. Organisms may also gain entry to the egg through shell damage such as cracks, which may be too small to be noticed. When this occurs bacteria are likely to be trapped by the shell membrane, and will usually only multiply in warm conditions. This would be expected to result in spoilage of the egg contents so that it would not be used for consumption as fresh egg, but there may be a period during which the bacterial population density may be insufficient to cause visual or olfactory abnormalities. Potentially zoonotic organisms (other than *Salmonella*) which may enter eggs in this way are listed below:

- *Staphylococcus aureus*
- *Escherichia coli*, including extended spectrum beta lactamase producers
- *Enterococcus/streptococcus spp*
- *Mycobacterium avium*

2.7 These and other organisms are considered to occur on the shell; examples of these “others” include:

- *Campylobacter spp.*,
- *Chlamydophila psittaci*
- *Bacillus cereus*
- *Listeria monocytogenes*
- *Toxoplasma gondii*
- *Erysipelothrix rhusiopathiae*
- *Clostridium perfringens*
- *Clostridium botulinum*

- *Aspergillus fumigatus* and other species
- *Proteus* spp
- *Pseudomonas* spp
- *Klebsiella* spp
- *Enterobacter* spp

- 2.8 Many of these would be considered to be spoilage organisms rather than zoonotic pathogens when considered in the context of eggs, but when present on the shell they may contaminate pooled egg dishes as a result of egg fragments and dislodged debris contaminating the pooled material. This would not normally lead to a problem if pooled liquid egg is kept refrigerated, but higher temperatures may lead to multiplication of pathogens and some organisms such as *Listeria monocytogenes* can still multiply, albeit slowly, at refrigeration temperatures.
- 2.9 Organisms originating from the surface of eggs can also contaminate industrial pooled egg products, either by direct contamination or contamination of the processing equipment and environment. *Listeria monocytogenes* in particular is able to form persistent biofilms on surfaces of equipment that can cause intermittent contamination of the product as it passes through the processing stages. The conditions for industrial processing and pasteurisation of egg, together with dilution of the concentration of micro-organisms when numerous eggs are pooled is thought to be largely effective in reducing risk, but process failures do occur which can lead to significant food poisoning outbreaks associated with processed liquid egg products.
- 2.10 Egg products are also susceptible to all the normal routes of contamination that apply for most foods, e.g. cross-contamination in the food preparation environment by transfer of pathogens from other food products, from infected or contaminated food, food handlers or from contaminated equipment. An example of this is likely to be *Campylobacter* infection linked with eggs.
- 2.11 Although some reports of studies in the USA record the findings of PCR products consistent with *Campylobacter jejuni* in hatching eggs in broiler hatcheries, epidemiological evidence suggests that egg-borne transmission is unlikely as *Campylobacter* survives poorly within eggs, does not readily multiply in eggs and survives poorly on shell surfaces, particularly under conditions of incubation, involving forced air at 35-37°C, found in broiler hatcheries. Although *Campylobacter* infection in broiler flocks tends to be seasonal, it is possible to control infection through good biosecurity in cold winter months, which would be impossible to do if egg-borne transmission was a significant component of the epidemiology, since all broiler parent flocks are infected with *Campylobacter* during the laying phase regardless of season. It would however

be possible for *Campylobacter* on the shell of very heavily contaminated eggs to be transferred to the hands of food preparation staff or to pooled egg dishes, but the level of contamination would be expected to be low and the opportunities for multiplication extremely limited, if possible at all. It is most likely therefore that the small number of *Campylobacter* outbreaks that have been linked with egg products have been wrongly attributed or have resulted from substantial cross-contamination.

- 2.12 Other pathogens such as *Bacillus cereus* and *Listeria monocytogenes* have been reported to be associated with foodborne outbreaks associated with egg products, particularly mixed food products containing eggs, rather than pure egg products. The origins of the contamination in such cases are unclear, but may originate from substances or sources other than the egg itself, or from contaminated catering equipment or personnel. The organisms may however also be present on egg shells and in egg processing facilities, so may ultimately have originated from eggs. Similar considerations also apply to other organisms which are common in the environment, such as *Staphylococcus aureus* or *Clostridium perfringens*.

Background on the public health risk associated with *Salmonella* in eggs and egg products:

- 2.13 *Salmonella* is the most commonly recognised bacterial species that can be vertically transmitted in poultry leading to the infection of broiler (chickens eaten for meat) chicks and sometimes the contents of table eggs. *Salmonella* Gallinarum and its biovariant *Salmonella* Pullorum are host-adapted to avians and zoonotic infection is not expected, and is extremely rarely reported (Anon, 2012). The turkey-pathogenic group K. *arizonae* serovar 018:z4, z23, is similarly transmitted in avians and rarely reported from humans (Hafez and Jodas, 2000). All of these serovars are not considered to be present in commercial scale poultry in GB, but *Salmonella* Pullorum may occasionally be found in backyard chickens or game birds, including commercially reared ones for shooting (Barrow and Neto, 2011). *Salmonella* Enteritidis, which is closely related to *Salmonella* Gallinarum, can also be transmitted vertically, but the efficiency of this varies with *Salmonella* Enteritidis strain, and will be discussed below. Other serovars such as *Salmonella* Heidelberg, *Salmonella* Infantis, and *Salmonella* Typhimurium may also be vertically transmitted to some extent, and this may be related to the fact that they can also spread from the gut to other organs, including the reproductive tract, by causing a systemic infection (Humphrey *et al.*, 1989a; Keller *et al.*, 1995; Okamura *et al.*, 2001a; Okamura *et al.*, 2010; Shivaprasad *et al.*, 2013). However, this is very variable according to the bacterial strain, the artificial infection protocols used and the

management of laying flocks. This will be addressed in Chapter 10 where the potential threat of *Salmonella* other than *Salmonella* Enteritidis is discussed.

- 2.14 In recent studies using comparative genome analysis, it was concluded that *Salmonella* Gallinarum and *Salmonella* Enteritidis are highly related and that the former may be a direct descendant of *Salmonella* Enteritidis, losing part of its genome in the process and becoming host adapted. Gene and associated surface antigen loss may be a way of allowing the pathogen to target particular tissues and can also affect its ability to survive in stressful situations within or outside the host (Thomson *et al.*, 2008; Feng *et al.*, 2013).
- 2.15 Other potential chicken-associated pathogens include a variety of viruses such as avian influenza. Although examples of human infection with poultry-associated viruses exist, there is no evidence that handling or consumption of table eggs is involved and infections are likely to be acquired through direct contact with infected poultry, usually via the respiratory route in a confined, dusty environment or in situations in which living accommodation is shared with infected poultry (Koopmans *et al.*, 2004). It is not our intention to discuss viruses any further in this report but to focus on *Salmonella* and *Salmonella* Enteritidis, in particular. There will also be brief discussion on other potentially human pathogenic bacteria that have been occasionally associated with eggs, which will appear later in this chapter. However, this report has *Salmonella* as its main focus.
- 2.16 In 2014, the European Food Safety Authority (EFSA) through its Panel on Biohazards published an authoritative report entitled “Scientific opinion on the public health risks of table eggs due to deterioration and development of pathogens” (EFSA 2014). This document contains much material relevant to this ACMSF report and we will refer to it. However, it is not our intention to duplicate what is in EFSA (2014) and we recommend those with an interest in the subject of eggs and public health to read the EFSA report.

***Salmonella* Enteritidis**

- 2.17 Overwhelmingly the most important zoonotic pathogen associated with eggs over the last 30 years across the world has been *Salmonella* Enteritidis. In the EFSA Report (2014) it is stated that: “*Salmonella* Enteritidis is considered the only pathogen currently posing a major risk of egg-borne diseases in the European Union (EU)”. Some of the bacterial attributes and virulence factors that make *Salmonella* Enteritidis a major threat to poultry and consumers through egg contents contamination will be discussed, mainly here and more briefly in chapter 10, to compare it with other *Salmonella* serovars. In our earlier

report (ACMSF 2005) we identified that the contamination of egg contents with *Salmonella* Enteritidis *in vivo* within the hen reproductive tract was the major reason for the international pandemic of egg-associated human infections with this serovar.

Mechanisms of contamination of eggs with *Salmonella* Enteritidis

2.18 The public health threat from *Salmonella* Enteritidis in eggs is largely related to its ability to be transmitted vertically. As with some other zoonotic pathogens, *Salmonella* Enteritidis can also contaminate egg contents by trans-shell transmission or during the breaking of the egg, when egg fragments or other debris from the surface of the egg or packaging may be dislodged into pooled egg (Todd, 1996). Further cross-contamination may occur at any stage during further processing from equipment, personnel or other food items added to the egg (Humphrey et al., 1994). Restriction of the impact of such secondary routes of contamination depends on general hygiene procedures and keeping the temperatures below that at which the micro-organisms can multiply, typically below 7°C for *Salmonella*, but *Listeria* and psychrotropic spoilage organisms can still multiply, albeit slowly, at refrigeration temperatures (see below).

Vertical transmission of *Salmonella* Enteritidis

2.19 Vertical transmission of *Salmonella* Enteritidis occurs within the reproductive tract of infected hens before the shell forms, largely due to the ability of the bacterium to persist and multiply long-term, possibly for the whole of the life of an individual hen, in the ovary or glandular tissue of the oviduct (Berchieri *et al.*, 2001). Deposition of *Salmonella* during the forming of eggs typically involves very small numbers of organisms (<10-20 cfu per egg) and is likely to preferentially occur within the albumen or yolk membrane. Direct contamination of the yolk is rare, but if this does occur *Salmonella* would be able to multiply in the nutrient-rich environment of the yolk. If this was a common route for egg contents contamination, more fresh naturally infected eggs would have been found with high numbers of *Salmonella* Enteritidis in them. This is a rare event (Humphrey 1994). Multiplication of *Salmonella* Enteritidis deposited in albumen or membranes is restricted by a large battery of inhibitory factors in the albumen and the scarcity of iron (Baron *et al.*, 2016). If *Salmonella* Enteritidis has infected the oviduct, organisms can be deposited into external layers of albumen, shell membranes or onto the shell, depending on the section of the organ which is colonised and timing of shedding of organisms into eggs (De Vylder *et al.*, 2013). Forming eggs produced by infected hens have been shown to have a much higher rate of contamination

than eggs after laying, suggesting that in many cases the low level of contamination introduced during egg formation does not survive (Keller *et al.*, 1995). To multiply, *Salmonella* Enteritidis must gain access to nutrients from the yolk, which happens over time as the yolk membrane begins to degenerate and become permeable. This happens more rapidly at higher storage temperatures and this membrane degradation may be followed by diffusion of yolk into the albumen and chemotaxis of *Salmonella* Enteritidis towards the yolk (Gross *et al.*, 2015).

- 2.20 There are several genetic factors that are involved in the process of infection of hen reproductive tissues and also in resistance to the inhibitory effect of egg albumen. The enhanced invasion of the reproductive tract and survival in the forming egg of *Salmonella* Enteritidis and *Salmonella* Gallinarum has been linked to the presence of SEF-14 fimbriae (Peralta *et al.*, 1994; Thiagarajan *et al.*, 1996; Rajashekara *et al.*, 2000; Rank *et al.*, 2009). *In vivo* gene expression technology revealed the *Salmonella* Enteritidis universal stress protein genes *uspA* and *uspB* were highly expressed in the chicken oviduct and eggs. Mutations in these genes compromised the ability to infect reproductive tissues and forming eggs (Raspoet *et al.* 2014).
- 2.21 Shah *et al* (2012) examined the role of *Salmonella* Enteritidis virulence genes in the infection of human gut epithelial cell lines, chicken liver cells and macrophages using transposon mutagenesis. Many genes were found to be important and some of these appeared to be almost specific to *Salmonella* Enteritidis. Raspoet *et al* (2014) used microarray detection to identify genes important in survival of *Salmonella* Enteritidis in primary chicken oviduct gland cells *in vitro* and persistence in the reproductive tract *in vivo*. Eighty genes were found to be important and major groups included those involved in stress responses and cell wall and LPS biosynthesis. Coward *et al* (2013) found that the expression of very long LPS O antigen in *Salmonella* Enteritidis is essential for egg contamination, probably linked with better infection of the reproductive tract and survival of the bacteria in egg albumen *in vivo*. In earlier work, Coward *et al* (2012) examined five pathogenicity islands in *Salmonella* Enteritidis, R1, 3, 4, 5 and R6. All played a small role in the infection of liver and/or spleen but not in the infection of the reproductive tract. Van Immerseel (2010) found that stress-induced bacterial survival strategies are important in allowing the persistence of *Salmonella* Enteritidis in hen reproductive tracts. McKelvey *et al* (2014) demonstrated that *Salmonella* Enteritidis antimicrobial peptide resistance genes were important in colonisation of the intestine and infection of the reproductive tract. Related work showed that in artificial infection studies the levels of *Salmonella* Enteritidis to infect the birds influenced the egg contamination patterns seen. Thus, Gast *et al* (2013)

showed that higher oral doses of *Salmonella* Enteritidis PT4 resulted in greater contamination of egg contents, with albumen being more likely to be positive than yolk.

- 2.22 Host factors are also important in the processes of egg contents contamination. Johnston et al (2012) found that loss of protective immunity systemically and in the reproductive tract when birds reach sexual maturity, even in vaccinated hens, increased susceptibility to *Salmonella* Enteritidis. The authors state that vaccination should be done in tandem with other measures such as improving bird welfare and biosecurity.

Horizontal transmission leading to internal contamination of eggs with *Salmonella* Enteritidis

- 2.23 One of the conclusions in our earlier report (2005) was that *in vivo* contamination of eggs with *Salmonella* Enteritidis in the reproductive tract of hens was a much more important public health threat than post-lay contamination of egg surfaces with faeces and other contaminated materials. However, we recognise that it is possible for micro-organisms, including *Salmonella* Enteritidis, to enter intact eggs if cooling takes place in a moist environment such that a negative pressure gradient is created that draws fluids and suspended micro-organisms into the egg. To gain access to eggs via the shell, *Salmonella* Enteritidis, or a few other micro-organisms, must traverse the cuticle, which has barrier and antibacterial properties, the eggshell itself and the shell membranes. The age and nutritional management of hens, as well as the disease status of the flock, can influence the quality of eggshells, and visibly abnormal eggs can indicate nutritional deficiencies or infection with certain viral diseases and mycoplasma infections (Roberts, 2004, Van Immerseel et al., 2011). Survival is prolonged in cool dry conditions and *Salmonella* Enteritidis is relatively resistant to desiccation and low temperatures and humidity restrict the competing activity of other organisms. In contrast, penetration of eggshells by *Salmonella* Enteritidis is enhanced at higher temperature and humidity, particularly if condensation is present on the eggs. The prevalence of shell contamination is always higher, usually by an order of magnitude, than internal contamination (Martelli and Davies, 2012).

Other possible egg-associated bacterial pathogens

- 2.24 A number of pathogens of potential public and animal health risk can be found in laying hens and other table egg-producing birds. A small number of these are considered to be truly vertically transmitted, which represents the biggest risk to public health, and can be found within egg contents, having been

deposited within the forming egg, including its shell membranes, during the development of the oocyte in the ovary or its transmission down the reproductive tract (Guard-Petter *et al.*, 2001). As discussed above, organisms, including *Salmonella* Enteritidis, may gain entry to the egg through the shell and this is exacerbated by damage such as cracks, which may be too small to be noticed (Messens *et al.*, 2005). When this occurs bacteria are likely to be trapped by the shell membrane, and will usually only multiply in warm conditions (Pouillot *et al.*, 2014). This would be expected to result in spoilage of the egg contents such that it would not be used for consumption as fresh egg, but there may be a period during which the bacterial population density may be insufficient to cause visual or olfactory abnormalities (Mayes and Takeballi, 1983; Svobodová, and Tůmová, 2014). As the above suggests, many of the above bacteria would be considered to be spoilage organisms rather than zoonotic pathogens when considered in the context of eggs, but when present on the shell they may contaminate pooled egg dishes as a result of egg fragments and dislodged debris contaminating the pooled material. This would not normally lead to a problem if pooled liquid egg is kept refrigerated, but higher storage temperatures may lead to growth of pathogens and some organisms such as *Listeria monocytogenes* can still multiply, albeit slowly, at refrigeration temperatures (Mahdavi *et al.*, 2012).

- 2.25 Organisms originating from the surface of eggs can also contaminate industrial pooled egg products, either by direct contamination or from processing equipment and environments (Musgrove *et al.*, 2004). *Listeria monocytogenes*, in particular, is able to form persistent biofilms on surfaces of equipment that can cause intermittent contamination of the product as it passes through the processing stages (Rivoal *et al.*, 2014). The conditions for industrial processing and pasteurisation of egg, together with dilution of the concentration of micro-organisms when numerous eggs are pooled is thought to be largely effective in reducing risk, but process failures do occur which can lead to significant food poisoning outbreaks associated with processed liquid egg products (EFSA, 2014).
- 2.26 Egg products are also susceptible to all the normal routes of contamination that apply for most foods, e.g. cross-contamination in the food preparation environment by transfer of pathogens from other infected or contaminated food, food handlers or from contaminated equipment.
- 2.27 *Bacillus cereus* and *Listeria monocytogenes* have been reported to be associated with foodborne outbreaks linked with egg products, particularly mixed food products containing eggs, rather than pure egg products. The sources of the contamination in such cases are unclear, but may originate from

substances or sources other than the egg itself, or from contaminated catering equipment or personnel. Similar considerations also apply to other organisms which are common in the environment, such as *S. aureus* or *Clostridium perfringens* (EFSA, 2014).

- 2.28 Cross-contamination of the shells of eggs can also occur on the farm or in packing plants at any stage during their collection, grading and packing through contamination of egg belts, brushes and general equipment. In practice, however, much of the surface contamination of eggs is removed by abrasion during these processes, resulting in a lower number of organisms present overall, even if more eggs become frequently contaminated (Davies and Breslin, 2003). Survival of such bacteria, including *Salmonella*, on the surfaces of eggs is dependent on the initial level of contamination and organic soil, temperature and humidity Shivaprasad *et al* 1990).
- 2.29 The EFSA report (2014) provides much detail on the relatively small number of bacterial outbreaks caused by pathogens other than *Salmonella* Enteritidis. However, the role of eggs or egg products in human infection with these bacteria is not clear and cross-contamination from other foods and/or equipment may be an important factor. It is not intended to repeat this detail here but the bacteria involved are *Bacillus cereus* and *Staphylococcus aureus*.
- 2.30 There has been discussion about the potential role of *Campylobacter* spp. as shell egg-associated zoonotic pathogens. *Campylobacter* is most frequently isolated from caeca, but is only rarely found on egg shells (Doyle, 1984; Shane *et al.*, 1986; Jones and Musgrove, 2007; Schwaiger *et al.*, 2008). Shane *et al.* (1986) demonstrated that *Campylobacter jejuni* cannot survive more than 16 hours in a dry environment.
- 2.31 Epidemiological evidence suggests that egg-borne transmission is unlikely as *Campylobacter* does not readily multiply in eggs (see below) and survives poorly on shell surfaces, particularly under conditions of incubation, involving forced air at 35-37°C, found in broiler hatcheries. Although *Campylobacter* infection in broiler flocks tends to be seasonal, it is possible to control infection through good biosecurity in cold winter months, which would be impossible to do if egg-borne transmission was a significant component of the epidemiology, since all broiler parent flocks are infected with *Campylobacter* during the laying phase regardless of season (Newell *et al.*, 2011). It would, however, be possible for *Campylobacter* on the shell of recently collected contaminated eggs to be transferred to the hands of food preparation staff or to pooled egg dishes, but the level of contamination would be expected to be low and the opportunities for multiplication extremely limited, if possible at all. It is most

likely therefore that the small numbers of *Campylobacter* outbreaks that have been linked with egg products have been wrongly attributed or have resulted from substantial cross-contamination.

- 2.32 The overwhelmingly important vehicle/source for human infection is contaminated chicken skin, and tissues, with undercooked liver, muscle and fluids associated with the carcass being especially important. *Campylobacter* infection rates in broiler flocks can reach 100 % (EFSA, 2010), although there is country-to-country variation with the EU average being ~75%. This pathogen can also be found in laying hen farms. There is a current debate on whether *Campylobacter* can be transmitted vertically and investigation/research has focused on broiler production.
- 2.33 In addition to being found in the intestine, *Campylobacter* is also able to colonize other organs and tissues such as the ovarian follicles and the reproductive tract (Cox *et al.*, 2009). Although the ability of *Campylobacter* to colonize reproductive tracts has been reported, the evidence for vertical transmission of the pathogen to eggs is equivocal. Some work suggests that it does not occur (Callicott *et al.*, 2006; Fonseca *et al.*, 2011). Sahin *et al* (2003) found that when *Campylobacter jejuni* was injected into the yolk of eggs it survived for up to 14 days but did not grow. Survival was poor in egg albumen or in the egg sac. With artificially infected SPF hens, *Campylobacter jejuni* was recovered from three of 65 pools of whole fresh eggs but not in one pool of eggs stored at 18°C for seven days before pooling. *Campylobacter* was not found in 500 eggs from naturally infected broiler breeders or from 1000 eggs from a commercial hatchery. Rossi *et al* (2012) used artificial infection studies of SPF hens infected with *Campylobacter coli* to examine the potential for vertical transmission. These authors conclude that while such transmission was possible by artificial infection, *Campylobacter coli* could not grow in commercial embryos surveyed from broiler breeders. There are some reports of studies in the USA where PCR products consistent with *Campylobacter jejuni* were found in hatching eggs in broiler hatcheries. However, the possibility of vertical transmission in broiler production is not widely accepted.
- 2.34 A study performed in Trinidad found that 1.1 % of sampled table eggs were contaminated by *Campylobacter* (Adesiyun *et al.*, 2005), and another study in Germany reported 4.1% of eggshells sampled were contaminated by this bacterium (Messelhäusser *et al.*, 2011). In the German study only egg shells were positive. In the EFSA Scientific Opinion on Quantification of the risk posed by broiler meat to human campylobacteriosis in the EU (EFSA BIOHAZ Panel, 2010c), around 1% of *Campylobacter* outbreaks were attributed to eggs, through source attribution based on outbreak investigation data.

However, Pires *et al.* (2010) questioned the reliability of using outbreak data for source attribution for *Campylobacter*, because the relative contribution of sources to sporadic and outbreak associated disease appears to differ, which could bias estimates based only on outbreak data. Fonseca *et al.* (2014), after a series of experimental contamination experiments concluded that commercial eggs do not represent a *Campylobacter* health hazard.

Eggs from other species

- 2.35 Production of table eggs from other species, such as ducks, quail, geese, turkeys, ostriches and seagulls, varies between countries and typically such eggs are sold as small-scale alternative, niche or luxury commodities. Improved standards, including egg date stamping and vaccination of birds against *Salmonella* are, however, being increasingly used by larger producers. Birds are typically housed on straw with floor-level nest boxes and open water troughs, which can lead to high litter moisture levels and faecal soiling of eggs. Eggs are normally collected manually several times a day and washed and/or bleached. This may remove the cuticle of the egg and theoretically make it more susceptible to trans-shell penetration, but chlorine bleaching appears to be highly successful in reduction of bacteria on shell surfaces, thus probably resulting in a lower overall risk (Martelli *et al.*, in press).
- 2.36 Duck egg production is typically less structured than the chicken egg industry (Huang and Lin, 2011), often with home breeding of birds, lower biosecurity standards and multiple age production, which limit the opportunities for all-in-all-out management. Moulting of birds is done frequently, or old birds from large producers may be sold on to smaller producers after a year of lay. Production of goose and turkey eggs is typically small scale, and eggs may be a by-product of small meat bird breeding flocks, although the public health risk associated with consumption of fertile eggs is increased (Kottwitz *et al.*, 2013). Flocks are normally floor housed on straw bedding in semi-open or free-range buildings on mixed livestock farms. Eggs are collected manually. Quail egg production may be more intensive, with birds housed either in floor pen systems or cages (Shanaway, 1994).
- 2.37 In most countries there is no formal *Salmonella* control programme for ducks, and infection may be common (Adzitey *et al.*, 2013; Cha *et al.*, 2013). As duck eggs are typically dirtier than hens' eggs when collected, there is a strong chance of faecal contamination of shells by other commonly occurring intestinal organisms, including *Campylobacter* and *Listeria* (Adzitey *et al.*, 2012). Internal contamination of eggs appears to be unusual (Nor Faiza *et al.*, 2013), although this may depend on the numbers of eggs examined and

hygienic conditions (Saitanu et al., 1994; Rezk and Saleh, 2008; Nor Faiza et al., 2013).

- 2.38 Outbreaks related to the consumption of duck eggs contaminated with *Salmonella* Typhimurium definitive phage type (DT) 8 have been reported in Great Britain, Northern Ireland and the Republic of Ireland (Noble *et al.*, 2012; Garvey et al., 2013). The link between human illness and duck eggs was supported by descriptive epidemiology and microbial evidence (Garvey *et al.*, 2013). The outbreaks were related (although definitive evidence could not be provided) to a breeding company persistently infected with *Salmonella* Typhimurium DT8, which was supplying infected day-old ducklings (Noble et al., 2012). *Salmonella* Enteritidis cases have also been reported (Nastasi *et al.*, 1998), but the duck-associated phage type (PT) 9b is very rarely reported in human cases.
- 2.39 Data on quail egg production are scarce and it is difficult to estimate the importance of these eggs across the EU. Quail egg production may be more intensive, with birds housed either in floor pen systems or cages (Shanaway, 1994). Young quail start to lay at around 6-12 weeks old and the eggs are typically collected by hand. Birds may have access to an outside veranda area and are often kept in groups of several thousand on shavings. Eggs are laid on the floor, but the birds tend to congregate in one area for egg laying. Laying birds are placed for 35-40 weeks, during which time each bird is expected to lay around 150 eggs. There is little information on the zoonotic risk associated with quail eggs, but *Salmonella* Enteritidis infection has been reported from some countries (Porter, 1998; Javan *et al.*, 2012; Katayama *et al.*, 2013). These data, like most of that relating to duck eggs, originate from Middle East or Far East countries, and are difficult to extrapolate to the current EU situation. Studies have shown that the potential for temperature-related growth, enhancement of contamination by temperature fluctuations and contamination of pooled egg dishes and the kitchen environment are similar for quails' and hens' eggs (Aikawa *et al.*, 2002). A low potential for trans-shell contamination and rapid die-off of shell contamination over time has also been observed (Katayama et al., 2013). *Listeria monocytogenes* can also be found on quails' eggs, leading to contamination of liquid egg (Erdogrul, 2004).
- 2.40 There is little information on zoonotic pathogens in laying geese flocks, but similar *Salmonella* infections to those found in ducks, as well as egg contamination, may occur (Yu *et al.*, 2008; Jahantigh, 2013). The same applies to turkeys, but *Salmonella* Enteritidis is very rare in turkey flocks and *Salmonella* from turkeys is considered to contribute little to human infection (EFSA BIOHAZ Panel, 2012; EFSA and ECDC, 2014). Ostriches are

normally housed outdoors, and *Salmonella* has been occasionally reported in flocks (de Freitas Neto *et al.*, 2009; Akbarmehr, 2010). It is apparent that the zoonotic risks associated with different species of poultry involved in small scale egg production are similar (Dale and Brown, 2013) and the risk in relation to eggs is highly influenced by the cleanliness of housing conditions, moisture levels and the effectiveness or otherwise of interventions such as egg washing.

Relevance of backyard chicken production

- 2.41 There is very little published information on the prevalence of *Salmonella* infection in small backyard chicken flocks in Europe, although occasional isolates are recovered from clinical investigation of such birds and some hot countries may present a higher risk (Pollock *et al.*, 2012, Manning *et al.*, 2015). It is recognised that backyard flocks may act as a reservoir of host-adapted *Salmonella* serovars, particularly *Salmonella* Pullorum (Waltmann & Horne, 1993), and that travelling farms involving young chicks from small breeding flocks may be a source of infection for children (Gaffga *et al.*, 2012). APHA field epidemiology studies carried out on mixed farms where backyard chickens are also present have normally found these small chicken flocks to be *Salmonella*-negative, even though they may be exposed to *Salmonella* Typhimurium from cattle, pigs or sheep. *Salmonella* Enteritidis has not been found in any backyard flock in such studies. It is likely that in small groups of birds the persistence of infection will be much less, since all birds will be exposed quickly and acquire a degree of immunity, whereas in large flocks there may be cycles of infection, clearance and re-infection that can perpetuate infection in a flock, as well as the occurrence of a very small percentage of birds that are persistently infected due to an inadequate immune response. Many pullets that are purchased for small flocks are of uncommon breeds, originating from small breeders that operate outside the *Salmonella* National Control Programme, and birds are not vaccinated for *Salmonella*. Some of those birds also originate outside the UK, from Eastern European countries when the risk of *Salmonella* Enteritidis may be greater than in Western countries. Birds purchased from mainstream pullet rearing companies are likely to be vaccinated, even if not declared as such, as birds are vaccinated *en masse* and it is difficult to obtain non-vaccinated commercial pullets in GB.
- 2.42 Another source of birds is re-use or rescue of spent hens. In commercial practice, hens will normally only be kept for laying, between 19 and 75 weeks of age, after which time they are functionally depleted and not able to produce economically within large flocks under commercial conditions. If the birds are removed to a less intensive environment they can recover feathering and continue to lay for another 1-2 years, producing predominantly extra-large eggs

that are favoured by consumers. If the original flock was infected with *Salmonella*, these older birds would present a significant risk, particularly if subjected to the stress of moulting (Holt, 2003), but such infection is unlikely in current British flocks.

- 2.43 Eggs from small flocks are not likely to be used in catering services but are thought to be used mainly within the family group who may already have gained some immunity to *Salmonella*, if present, through association with the birds, servicing their pens and contact with faecal matter, as well as heavily contaminated eggs (although the impact of internet and small market sales is currently unclear). It is likely that such residual immunity due to ongoing exposure may also be under-estimated in the general populations of egg consumers. Eggs from small flocks are likely to be collected very fresh and may be dirtier than most commercially produced eggs, so if infection is present there may be more opportunity for contamination of the kitchen as eggs will be taken straight from the nest to the kitchen with minimal storage. Timely consumption of eggs will also minimise the opportunity for multiplication of internal contamination.
- 2.44 There may be more risk associated with eggs from small flocks that operate on a semi-commercial basis but fall outside the requirements of the NCP. Such eggs may be stored at ambient temperatures and sold at the farm gate with minimal attention to hygiene. These flocks may also include old birds that are more likely to harbour infection and the flocks are often of multiple ages, with no break in production in which to eliminate infection if it is present. A survey of flocks of between 100 and 350 birds would be necessary if this risk was required to be quantified, but this type of production still only generates a very small proportion of British Eggs, and so the total impact is likely to be small even if the risk per egg was found to be greater.

Microbial contamination of egg products

- 2.45 The manufacturing process for egg products is not a sterile operation and the raw material in terms of eggs' shells, if not contents, is inevitably contaminated by a range of bacteria. Eggs from flocks that are infected with *Salmonella* Enteritidis or *Salmonella* Typhimurium are directed for processing in many countries. Similarly, second quality eggs with shell defects and surplus fertile hatching eggs, which are at increased risk of contamination, are normally sent for processing. Small eggs from laying flocks at the onset of lay and poor quality eggs from birds at the end of lay may also be more at risk from contamination if *Salmonella* is present in the flock.

- 2.46 The European egg product industry mainly purchases eggs directly from farms or from packing centres. Regulation (EC) No 853/2004 also authorises the processing of eggs downgraded by packing centres, including cracked eggs or eggs produced on farms contaminated with *Salmonella*. Cracked eggs and eggs provided by contaminated farms must be broken and pasteurised upon arrival in egg processing plants. Information received from the egg industry indicates that in most countries table eggs that have reached their 'sell-by date' at retail are not diverted to egg products, as this practice is rarely economically viable. When such practices do occur, they are small in scale. According to the current legislation, there is no 'best before date' for eggs destined for the production of egg products.
- 2.47 Commission Regulation (EC) No 853/2004 defines egg products as 'processed products resulting from the processing of eggs, or of various components and mixtures of eggs, or from the further processing of such processed products'. Processing consists of 'any action that substantially alters the initial product, including heating, smoking, curing, maturing, drying, marinating, extraction, extrusion or a combination of those processes', as described in Commission Under regulation EC No 852/2004, egg products from 'first processing' are defined as resulting from the breaking of table eggs, giving rise to the recovery of whole egg or separated egg yolk and egg white, with the possible addition of salt, sugar and hydrocolloids (Lechevalier et al., 2011). They are mainly delivered in the form of refrigerated liquid egg, but also as frozen or dried powder products.
- 2.48 Egg products are widely used for various food applications, suitable for artisans, catering and as ingredients for the food industry, being used in sauces, pasta, biscuits, cakes, processed meats, fish products, wine products, ice creams and refrigerated desserts. In some countries, 'first processing' egg products may also be available for consumers, e.g. as pasteurised liquid egg for body-builders or home cooking.
- 2.49 In addition to egg products from 'first processing', which are by far the predominant products of the egg-processing industry, 'speciality' egg products are also manufactured. These products result from the cooking of either table eggs or formulated egg products (whipped egg whites, poached eggs, scrambled eggs, hard-boiled eggs, pickled eggs, fried eggs or omelettes). They are sold either directly to consumers or through mass catering.
- 2.50 Pasteurisation of liquid egg is an important public health intervention. In the EU, whole egg and egg yolks are heat treated at temperatures of 65 to 68°C for 5 to 6 minutes. Egg whites receive milder treatments (55–57°C for 2–5

minutes) (Baron and Jan, 2011), due to the greater heat sensitivity of egg white proteins. These treatments are adequate to reduce the vegetative microflora by at least 6 logs in whole eggs or egg yolk (Baron *et al.*, 2010). However, they are ineffective against the heat-resistant microflora, including spore-forming bacteria. These organisms are, however, unlikely to be involved in food-borne outbreaks due to the consumption of egg products. It is estimated that around 1% of bacteria survive the pasteurisation step, but these are largely more heat-resistant or spore forming organisms such as *Streptococcus*, *Enterococcus*, and *Bacillus*. *Bacillus* species, in particular can lead to substantial spoilage and economic loss in egg processing. *Bacillus* and other bacteria such as *Listeria* can also form biofilms on the stainless steel egg processing equipment and there may be multiplication of psychrotolerant toxigenic species/strains such as *Bacillus weihenstephanensis*, during subsequent storage. Gram-negative organisms can survive inadequate pasteurisation, or recontaminate pasteurised products. Such recontamination events may also lead to foodborne outbreaks of *Salmonella* associated with inadequately treated liquid egg products, especially if these are consumed without further cooking, although such cases have only been from EU sourced eggs. In GB, laying flocks infected with *Salmonella* Enteritidis are slaughtered, so the entry of contaminated eggs into processing establishments should be low compared with other countries in which eggs from infected flocks are routinely diverted for heat treated product.

- 2.51 There appears to be little up to date information on the microbial contamination of dried egg. Some more heat-resistant organisms that survive pasteurisation of liquid egg may also survive the drying process. It is also possible that the dried egg might become contaminated post-drying. However, the organisms that survive the drying process or are post-drying contaminants are not likely to multiply in dried egg (Santillana *et al*, 2014).

Conclusions

- 2.52 Only *Salmonella* is a relevant zoonotic pathogen of fresh eggs. Other bacteria such as *Listeria monocytogenes* or *Bacillus cereus* may contaminate egg processing equipment and lead to contaminated products if processing errors occur, but this hazard does not relate to the eggs themselves
- 2.53 Of the zoonotic pathogens, only *Salmonella* Enteritidis, and within that serovar only certain strains, are able to persist for long periods in the reproductive tract of hens and cause internal contamination of eggs.
- 2.54 Shell contamination is more common than internal contamination and can involve various serovars. It is considered that shell contamination represents a

much smaller risk than internal contamination, but this has never been quantified and further work would be required to investigate this

- 2.55 It is most likely that the small numbers of *Campylobacter* outbreaks that have been linked with egg products have been wrongly attributed or have resulted from substantial cross-contamination.
- 2.56 Commercial eggs do not represent a *Campylobacter* health hazard.
- 2.57 *Salmonella*, particularly certain genovars of *Salmonella* Enteritidis, are by far the most significant pathogens that are primarily associated with eggs. This is because of the ability to colonise the avian reproductive tract on a long-term basis leading to intermittent shedding of the organism into the contents of forming eggs.
- 2.58 Other organisms may be present on the surface of eggs and be transferred to the egg processing environment, but are no more risk in association with eggs than for other foods eaten raw.
- 2.59 Monitoring and control of *Salmonella* in egg laying species other than chickens is relatively poorly developed, so the overall risk is unknown and it would not be advisable to relax current guidance of cooking of such eggs.
- 2.60 There are insufficient data available to assess the risk of *Salmonella* infection in small flocks that are not included in the NCP monitoring and the risk associated with eggs, but this is thought to be low and the impact on the population as a whole is extremely low.
- 2.61 Although egg products are pasteurised, it is possible for treatment failures and recontamination to occur, but the risk applies primarily to imported egg products since in the mainland UK, eggs from positive flocks are not diverted to processing into egg products, whereas in many other EU countries this is normal practice.

Chapter 3: Egg industry in the UK. Consumption patterns relating to different egg types and products

UK egg consumption (Hens' eggs)

- 3.1 The UK is the sixth largest egg producer in EU. There are approximately 34.8 million laying hens in the UK. The UK hens' egg market comprises barn, organic, enriched cage and free range eggs. Enriched cage and free range eggs account for the majority of the market share. Table 2 illustrates the UK market share of different types of hens' eggs.

Egg production method	Market share (%)
Enriched cage	52.0
Free range	42.8
Barn eggs	2.9
Organic	2.3

Table 2: 2014 UK market split by volume of different egg types
(Source: DEFRA)

- 3.2 In 2014, it was estimated by the egg industry that approximately 11.8 billion eggs were consumed in the UK per annum (184 per Capita and 32 million per day). Egg sales were estimated to equate to £955 000 000. The UK egg market can be divided into retail (52.5%), food manufacture (23%) and food service (24.5%). It was estimated by the egg industry that 1,898 million eggs were imported into the UK in 2014 and 134 million eggs were exported from the UK. <https://www.egginfo.co.uk/egg-facts-and-figures/industry-information/data>.

UK egg production systems

- 3.3 Across the EU, conventional 'battery' cages have been banned. In the UK, they have been replaced by larger, 'enriched' colony cages. The EU egg marketing legislation requires that for eggs to be termed 'free range', hens must have continuous daytime access to runs which are mainly covered with vegetation and a maximum stocking density of 2,500 birds per hectare. The hen house conditions for free range hens must comply with the regulations for birds kept in barn systems, with a maximum stocking density of 9 hens per square metre of useable area. In barn systems, hens are able to move freely around the house. The EU Welfare of Laying Hens Directive requires a maximum stocking density of 9 hens per square metre of useable floor space. Hens producing organic eggs are always free range. In addition, such hens must be fed an organically produced diet and ranged on organic land.

- 3.4 The hen house conditions for organic hens are set by the EU Organic Regulations and require a maximum stocking density of 6 hens per square metre of useable area and a maximum flock size of 3,000 birds (Source: BEIC).
- 3.5 Approximately 90% of UK eggs are now produced under the Lion Code Quality Assurance scheme <http://www.britisheggindustrycouncil.co.uk/Home/>. This scheme covers the entire production chain and incorporates specific food safety controls. The scheme requires vaccination of hens against *Salmonella* Enteritidis and *Salmonella* Typhimurium, registration and a unique 'passport' system, ensuring complete traceability of hens, eggs and feed. Increased hygiene controls and additional *Salmonella* testing in the integrated egg production chain, including turnaround post disinfection swabbing of breeding, pullet rearing and laying flocks and packing centre hygiene testing are amongst others additional requirements for the scheme.

Egg products

- 3.6 In simple terms egg products can be described as eggs which have been processed and packaged into a more convenient form (see below for full regulatory definition)³. A number of egg products are available on the UK market (refrigerated liquid egg, frozen egg, dried egg and cooked egg products). Although historically, eggs were marketed primarily as boxes of shell eggs, in recent years egg products have become increasingly popular as their consumption in domestic and commercial settings has increased (Source: BEPA). In 2001, the volume of egg products was 1.9bn eggs equivalent; in 2014, the volume was 2.6bn eggs equivalent, which means the market, has grown by approximately 40% since 2001. The largest growth in the use of egg products has been in the service industry where such products are relied on for their convenience often being purchased as pre-measured ready-to-use products. Most egg products are pasteurised. Information on frozen eggs was difficult to obtain.
- 3.7 During 2013, the production of liquid egg and egg products totalled 95,005 tonnes (62,338 tonnes liquid egg and 32,667 tonnes egg products). The corresponding 2014 figures were 101,878 tonnes (66,910 tonnes liquid egg and 34,968 tonnes egg products). (Source: DEFRA, 2015).
https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/451601/eggs-statsnotice-07may15.pdf

³ Egg products are defined in Regulation (EC) 853/2004 as “processed products resulting from the processing of eggs, or of various components or mixtures of eggs, or from the further processing of such processed products.”

Consumption of eggs other than hens' eggs in the UK

Duck eggs

- 3.8 Information relating to duck eggs was more difficult to obtain. There are approximately 100,000 egg laying ducks in the UK, mainly linked to three main producers. If it is estimated that each duck lays 300 eggs / bird / year, it can be estimated that around 2.5 million dozen eggs are produced annually (ADAS, personal communication).

Quails eggs

- 3.9 Data relating to the quails' egg market is perhaps the most limited of all. Data from a limited number of sources have estimated that the quail egg market share is approximately 170 000 eggs a week.

Internet sales of eggs

- 3.10 Data on internet sales are not available.

Conclusions

- 3.11 The UK is the sixth largest egg producer in EU. There are approximately 34.8 million laying hens in the UK. The UK hens' egg market comprises barn, organic, enriched cage and free range eggs. Enriched cage and free range eggs account for the majority of the market share.
- 3.12 In 2014, it was estimated by the egg industry that approximately 11.8 billion eggs were consumed in the UK per annum (184 per Capita and 32 million per day). Egg sales were estimated to equate to £955 000 000.
- 3.13 Approximately 90% of UK eggs are now produced under the Lion Code Quality Assurance scheme.
- 3.14 A number of egg products are available on the UK market (refrigerated liquid egg, frozen egg, dried egg and cooked egg products). Although historically, eggs were marketed primarily as boxes of shell eggs, in recent years egg products have become increasingly popular and their consumption in domestic and commercial settings has increased by 40% since 2001.
- 3.15 Information relating to duck eggs was more difficult to obtain. It can be estimated that around 2.5 million dozen eggs are produced annually
- 3.16 Data relating to the quails' egg market is perhaps the most limited of all. Data from a limited number of sources have estimated that the quail egg market share is approximately 170 000 eggs a week.

3.17 Data on internet sales are not available.

Recommendation

3.18 We recommend that data relating to internet sales of different types of eggs are gathered by the most suitable means to determine the extent to which internet sales influence the UK egg market.

Chapter 4: Storage, handling and use of eggs

Introduction

- 4.1 Despite considerable improvements in the microbiological safety of eggs over recent years, it is still possible for some eggs entering catering and domestic environments to be contaminated with *Salmonella*. It therefore remains important that eggs and egg products are carefully stored, handled and used in these environments.

Egg production and consumption

- 4.2 In 2014 the UK produced over 9.75 billion eggs, of which 134 million were exported. During the same period, a further 1.89 billion eggs were imported. Overall almost 12 billion eggs consumed within the UK in 2014, i.e. 32 million per day, and an average of 184 per person per year. This means that the egg consumption per person has more than doubled within the last 15 years. Egg sales in 2014 were estimated to equate to £955 M, with 52.5% entering the retail food chain, 23% going into food manufacture and 24.5% being used in food service/catering. Most retail eggs (88%) were sold through the major multiples, although other outlets include co-ops, market stalls, independent shops, butchers and “others” <https://www.egginfo.co.uk/egg-facts-and-figures/industry-information/data>

Food safety concerns

- 4.3 The principal food safety concerns in relation to the use of eggs in catering and domestic food production include failure to observe “best before dates”, inappropriate storage including temperature abuse, bulking of eggs, cross contamination during food preparation and production, and the consumption of raw eggs (or undercooked dishes containing raw eggs). The latter practice poses particular risks to higher risk/vulnerable groups, i.e. (the very young, the very old, the pregnant or the immunocompromised). Some of these concerns are of greater or lesser relevance to the above sectors, for example, issues around bulking and use of liquid egg is more relevant to larger scale catering and food production environments.
- 4.4 The mechanisms by which raw shell eggs may become contaminated with *Salmonella* are discussed at length in other parts of this report. However there are a number of points of particular relevance in relation to the use of eggs in catering, retail or domestic food production.
- 4.5 Eggs may become contaminated with *Salmonella*, internally during the egg development process, or externally, by contamination within the hen’s reproductive tract, or contact with faeces after lay (Humphrey *et al*, 1994,

Humphrey 1994). External contamination can travel through the egg shell, especially just after laying or if eggs are stored in humid conditions (de Buck *et al*, 2004).

- 4.6 External contamination present on the outer surface of the eggs poses risks in relation to cross contamination of the egg contents, during handling and use, as well as cross contamination of hands, utensils, work surfaces and other foods (Kramer *et al*, 2006, Carrasco *et al*, 2012). The well-recognised abilities of *Salmonella* to persist on, and spread from, such sources (De Oliveira *et al*, 2014, Wang *et al*, 2015) reinforce the importance of effective food hygiene training programs in relation to the correct handling of eggs and foods containing eggs, effective cleaning, and the avoidance of cross contamination within food processing activities. Similarly potential pathogens on other foods such as chicken and raw meat can cross-contaminate eggs and this could be a particular risk with pooled egg. While much of this training focuses on food handlers in larger scale food processing and catering kitchens, the knowledge and good practice advice provided within such training is likely to be of value to any and all domestic food handlers, especially if they may be involved in the preparation of food for higher risk groups or individuals.
- 4.7 Temperature abuse allows any *Salmonella* present to multiply rapidly in whole egg contents once the yolk membrane has degraded sufficiently to allow the bacteria to the nutrient-rich yolk contents. Thus care is needed to ensure that eggs are appropriately stored, under refrigerated conditions. Recent EFSA advice (2014) confirms the importance of effective refrigeration (retail, catering and domestic) in maintaining overall physiochemical properties and microbiological safety/quality of stored eggs (EFSA, 2014). However, as noted above, wet egg shells can allow *Salmonella* to penetrate into egg contents (EFSA, 2009), so it is important to limit condensation on eggs after removal from chill storage. A recent study has confirmed the value of cooling in relation to limiting *Salmonella* growth in eggs (Gross *et al*, 2015), and FSA advice remains that eggs should be stored in the refrigerator below 8°C in catering, food production and domestic premises.
- 4.8 The above EFSA opinion notes the particular importance of effective refrigeration in relation to the storage of pooled eggs. The risks associated with the use of pooled eggs relate to the potential that eggs containing *Salmonella* may contaminate other eggs during the pooling/bulking process, and subsequently grow in the absence of effective refrigeration, with the potential of affecting a large number of consumers. Any food poisoning bacteria (including *Salmonella*, but excluding bacterial spores) will be killed by thorough cooking of eggs to an internal temperature of 70°C for 2 minutes, but any uncooked food products containing contaminated pooled eggs pose increased risks.

- 4.9 Consumer preferences in relation to ethical food production and sustainability have increased the extent to which caterers seek to source and serve organic and free range eggs. Similarly, shifts in consumer appetite for unprocessed catering/home-made foods containing raw eggs including mayonnaise and sauces, ice cream, milk-shakes and tiramisu, increased the risk of salmonellosis from such dishes in the UK before the industry introduced comprehensive control measures such as the Lion Code. Such risks were also increased by changes in cooking and serving practices within commercial and domestic kitchens, associated with low temperature cooking and the deliberate serving of food products in a raw or rare state (ACMSF, 2013). Such inadequate cooking, in which foods are treated at time temperature combinations which fail to reduce numbers of pathogens of concern to an acceptable level, means that the risks posed by *Salmonella* and other bacteria present in eggs and egg products may persist, if eggs are obtained from sources that do not include the comprehensive measures introduced in the UK under the Lion Code. It is the view of the Working Group that if caterers use eggs sourced from producers operating under the Lion code, eggs can be used uncooked but dishes must be protected from cross-contamination from other potential sources of *Salmonella* and other food borne pathogens. We support FSA advice that caterers should use pasteurised egg for any food which is likely to be served uncooked, or lightly cooked, if 'Lion code' eggs are not used (FSA, 2002).
- 4.10 In 2002, a pilot study to estimate the nature and extent of adherence to government guidance on safe egg use in the catering industry reported that there was little awareness of food safety risks associated with eggs and that recommended good practice is not widespread (Taylor, 2004). In 2006 a UK wide survey observed continuing poor practice in relation to egg storage and handling practices in catering premises, with 55% of eggs not stored under refrigerated conditions or more than 20% of eggs had expired "best before" dates (FSA, 2007). Overall 37% of the surveyed catering premises mixed and pooled shelled eggs, and this practice was even more frequent (47 – 50%) in institutional, hotel and restaurant kitchens. Not all pooled eggs were stored under chilled conditions. It is not clear how far things have progressed since that time.
- 4.11 The FSA provides advice to caterers on safe handling of eggs (FSA, 22/08/14, 2014) in relation to:
- keeping eggs away from other foods, when they are still in the shell and also after they have been cracked open;
 - taking care to avoid splashing raw egg onto other foods, surfaces or dishes during bulk "pooling" (the process of breaking eggs to use later;

- keeping bulk liquid egg in the fridge and only taking out small amounts as needed;
- using of all 'pooled' liquid egg on the day of pooling;
- avoiding adding new eggs to stored bulk eggs;
- cooking eggs and foods containing eggs thoroughly;
- using pasteurised egg for raw or lightly cooked foods;
- always washing and drying hands thoroughly after touching or working with eggs;
- cleaning food **areas**, dishes and utensils thoroughly and regularly using warm soapy water, after working with eggs;
- serving egg dishes straight away, or cooling them quickly and keeping them chilled.

Domestic use of eggs

- 4.12 There remains very little information on how consumers handle shell eggs in domestic settings, although it is known that some consistently underestimate perceived personal risk in relation to safe storage, handling and cooking of food (Kennedy *et al*, 2005, Meah, 2013). Such misunderstanding persists despite considerable efforts to increase public understanding of the value of using safe food handling practices in the home (Taylor, 2004).
- 4.13 The egg industry has provided advice on domestic eggs storage and handling is provided by "egg info" <https://www.egginfo.co.uk/egg-safety/storage-and-handling> including advice in relation to selecting eggs from vaccinated hens, low temperature storage, hand hygiene and avoiding dirty, cracked or broken eggs.
- 4.14 The overall risks of consumers becoming infected after consuming a *Salmonella*-contaminated egg increases as the egg gets older so careful adherence to "Best before Dates", where available, can help reduce the risks that any *Salmonella* present will cause food poisoning.
- 4.15 Irrespective of the source of eggs, particular concerns remain where catering for larger functions is carried out in domestic premises where facilities for proper storage, temperature control and cooking, as well as the avoidance of cross contamination are often inadequate or inappropriate. Failure to take basic food hygiene measures including refrigerated storage, thorough cooking and avoiding cross contamination increases the risks of human salmonellosis, especially when 'non-Lion code' eggs are used. Even when 'Lion code eggs' are used it is particularly important to ensure that dishes that will not receive cooking are protected from cross-contamination from potential sources of *Salmonella* and other food borne pathogens that are able to grow in raw egg.

- 4.16 While the risks posed by such materials are much lower within the UK than in other parts of the world, it is important to remember the higher risk that they pose to susceptible groups within the UK population.

Recent changes

- 4.17 A number of changes in sourcing, production and consumer preferences have become more significant since the ACMSF's Second Report on *Salmonella* in Eggs (2001) which increase risks in relation to unregulated eggs and egg products. These include:

Non-UK eggs

- 4.18 Prior to the EU-wide implementation of the *Salmonella* NCP in laying chicken flocks in 2008, the conditions under which non-UK eggs were produced, and subsequently traded into the UK, differed widely between different exporting countries and were not comparable with the conditions under which UK eggs were produced. Such differences could have resulted in non-UK eggs posing greater risk of the development of human salmonellosis. For example, following Health Protection Agency investigations of a series of outbreaks of food poisoning associated with Spanish eggs (2002-2004), FSA issued specific advice to caterers that all eggs from Spain should be heat-treated before use. Widespread implementation of this advice led to a sharp fall in the number of outbreaks of salmonellosis associated with Spanish eggs.
- 4.19 However, following the implementation of Regulation 2160/2003, from 2008 new harmonised *Salmonella* monitoring and control requirements for egg laying flocks in all Member States were introduced (the *Salmonella* NCPs). The requirements included, as a minimum, the harmonised sampling and testing requirements laid out in the annex to Regulation (EC) No. 517/2011 (amending the original Regulation 1168/2006) and minimum harmonised risk mitigation measures also apply in every Member State. There has been significant progress made in reducing *Salmonella* prevalence in the commercial egg production sector across the EU but especially in some of the Member States from whom the UK has sourced or currently does still source eggs, although the UK still has significantly lower flock prevalence based on the harmonised statutory monitoring (Table 1). From 2008, for third countries wishing to export eggs to the EU, Article 10 of Regulation (EC) No. 2160/2003 requires that the country must implement equivalent measures for the control of zoonoses and have in place a *Salmonella* National Control Programme approved by the EU Commission. Very few third countries currently have approved programmes and therefore are able to export eggs to the EU. Therefore, the situation after 2008 is not directly comparable to that in previous years prior to the introduction of the *Salmonella* NCPs.

4.20 As noted earlier in this section, although the UK exports some of the eggs produced here, it also imports eggs, mainly destined for the catering industry. EU and worldwide data continue to confirm the presence of *Salmonella* in eggs in many countries (EFSA/ECDC, 2015), suggesting the need for particular care in handling and using eggs and egg products from countries that still have a higher prevalence of *Salmonella* Enteritidis in primary production than the UK .

Eggs and egg products on-line

4.21 A wide range of eggs, especially speciality ones, are becoming widely available and easily accessible on-line e.g. free-range/organic/duck/turkey/quail/goose/ostrich/emu/ pheasant, etc. are available for purchase on-line. Similarly, a range of egg products e.g. whole liquid egg and liquid egg whites can be purchased on-line. The latter mostly aimed at the catering sector (or body builders) are usually pasteurised, especially if they are produced within the UK. However, it is less clear how internet based sales of eggs and egg products can be adequately monitored, and the quality of such products assured.

Small holding/backyard egg production

4.22 Under current legislation, all poultry flocks of more than 50 birds must be registered on the central register containing poultry population data required for the prevention, control and risk assessment of poultry disease in Great Britain and Northern Ireland. Registration is voluntary if fewer than 50 birds are kept on the premises, so although some information is available for premises in the UK with fewer than 50 birds based on this voluntary registration, not all flock owners register voluntarily. Specifically for the laying chicken sector, all Class (Grade) A eggs sold at retail outlets and public markets within the EU must be stamped with a code which identifies the method of production, country of origin and establishment number except where eggs are sold directly to the consumer for their own use through farm gate sales, doorstep sales or in a local market. Small scale producers with fewer than 50 birds are not required to mark eggs with a producer code, but if the eggs are sold at a market, producer name, address, the best before date and advice on how to keep eggs chilled after purchase must be displayed. For small scale producers with 50 or more hens, there is a requirement to be registered and eggs must be stamped with the producer code and best before date and advice provided to keep eggs chilled after purchase. For larger commercial producers, there is additional legislation, the Registration of Establishments (Laying Hens) (England) Regulations 2003 and equivalent legislation in the Devolved Governments, which requires all producers with a laying hen establishment of 350 or more laying hens to register and provides for provision of this information to public health authorities where this is necessary to trace eggs put on the market for human

consumption. If eggs are marketed to shops or catering outlets, the producer must be approved and authorised as a packing centre so that eggs can be graded as Class A eggs.

- 4.23 The requirements of the *Salmonella* NCP apply to all operators who produce eggs on a commercial basis, with only two exceptions: where all production is for private domestic use only (i.e. the eggs are not entering the market) or where the producer has fewer than 350 hens and only supplies eggs direct to the consumer (i.e. farm gate sales) or via local retailers that only supply the final consumer (essentially householders). The definition of 'local' is according to current Food Standards Agency guidance which specifies local as the supply of food of animal origin within the supplying establishment's own county plus the greater of either the neighbouring county or counties or 50 km/30 miles from the boundary of the supplying establishment's county.
- 4.24 The basis of the exemption from the requirements of the NCP is defined in the EU legislation Regulation 2160/2003 Article 1 and was included as it was considered at the time that small scale production did not make a significant contribution to the average prevalence of zoonoses in animal populations in the Community as a whole (i.e proportionate to risk), the general requirements for sampling and analysis may not be practical or appropriate in very small poultry flocks and that traceability of product was facilitated through the 'local' nature of product distribution. Member States are required to establish national rules for this small scale production, where considered necessary for the protection of public health. For the UK, these national requirements are as specified above. This means that eggs produced in such micro-flocks fall outside the harmonised protections provided by the current regulations, standard and production requirements.
- 4.25 The duck sector, irrespective of the size of production, is not subject to mandatory NCP and does not fall within the scope of Regulation (EU) 2160/2003. These small sectors currently represent a relatively small element within the UK egg production, processing and consumption chain, but may become more significant if increasing trends continue. It may be worthwhile to collect data on the production, use and consumption of duck/other poultry eggs and *Salmonella* occurrence in order to be better placed to assess the public health risk.

Conclusions

- 4.26 EU and worldwide data continue to confirm the presence of *Salmonella* in eggs in many countries (EFSA/ECDC, 2015), suggesting the need for particular care in handling and using eggs and egg products from countries that still have a higher prevalence of *Salmonella* Enteritidis in primary production than the UK .

- 4.27 While outbreaks of food poisoning associated with the consumption of eggs continue to present significant public health challenges in other parts of the world, including other parts of the EU and the US, such outbreaks linked to domestically produced eggs are much less frequent within the UK since the previous ACMSF report on *Salmonella* in Eggs (2001). Since 2009, the majority of general outbreaks of foodborne disease linked to eggs and/or egg products for which an origin of the eggs could be identified, were linked to imported eggs.
- 4.28 Over the 7-year-period from 2008 – 2014, despite the overall increase in reported cases in 2014, there was a statistically significant decreasing trend for salmonellosis in the EU with significantly decreasing trends in nine MS (Belgium, Cyprus, Denmark, Finland, Germany, Ireland, Slovakia, Sweden and the United Kingdom). There may be a number of reasons for these reductions. They are however, very likely to be associated with a number of specific legislative and industry based interventions. Such reductions in the incidence of human salmonellosis cases within the UK and the EU, have been attributed, at least in part, to control of *Salmonella* in the broiler, laying and breeding hen flocks, and in eggs (Defra, 2010).
- 4.29 However, there remains a potential for the occurrence of cases/outbreaks of salmonellosis within the UK, associated with the consumption of un/undercooked eggs, which have not been produced, processed, and distributed in line with current best industry practice, and robust public health controls. Such eggs will continue to pose risks, especially among higher risk/more susceptible groups within the overall population.
- 4.30 Those involved in the storage, handling and use of eggs should therefore be aware that the risks in relation to *Salmonella* in eggs are significantly affected by egg sources and history and poor hygiene and preparation practices. The risks associated with eggs produced and distributed within an appropriate comprehensive quality and safety management system such as that provided by the British Lion mark certified farm assurance scheme are likely to be lower than the risks associated with eggs which are not produced in, and protected by, such systems.

Recommendations

- 4.31 We recommend reinforcement of good hygiene guidance and training in all settings.
- 4.32 We recommend that up-to-date information relating to catering practices, such as pooling and storage of eggs, is obtained.

- 4.33 We recommend that trends in the duck egg market are monitored and consideration given to exploring the extension of existing NCP regulation to ducks. As part of this we also recommend monitoring of *Salmonella* occurrence in these eggs to be better placed to assess the public health risk.
- 4.34 FSA advice remains that eggs should be stored in the refrigerator below 8°C in catering, food production and domestic premises.
- 4.35 Consumer preferences for unprocessed catering/home-prepared food containing raw eggs, along with deliberate undercooking of such foods increases the risk posed by *Salmonella* with eggs not sourced from schemes with a comprehensive suite of control measures like the UK Lion Code, or schemes equivalent to it. Eggs from any source should be protected from cross-contamination by any potential food poisoning bacteria. The Working Group supports FSA advice that if caterers do not purchase eggs produced under the Lion Code or a scheme equivalent to it, they should use pasteurised egg for any food which is likely to be served uncooked, or lightly cooked.
- 4.36 Two studies in the catering industry (2002, 2006) have identified poor practice in relation to egg storage and handling, including pooling of eggs, in catering. FSA should review the uptake/impact of the most recent FSA advice in this area (2014).
- 4.37 FSA should ensure that those involved in the storage, handling and use of eggs know that the risks in relation to *Salmonella* in eggs are significantly affected by egg sources and history, specifically, the risks associated with eggs produced and distributed within an appropriate comprehensive quality and safety management system such as that provided by the British Lion mark certified farm assurance scheme are likely to be lower than the risks associated with eggs which are not produced in, and protected by, such systems.

Chapter 5: Description of interventions relating to laying hens, chickens, ducks quails and any other

(a) Interventions to control *Salmonella* in egg production

- 5.1 The dramatic increase in *Salmonella* Enteritidis in the early to mid-1980s led to changes in surveillance such that it became a legal requirement to report all *Salmonella* isolates to agents of the competent authority and a requirement to monitor commercial chickens breeding flocks and flocks of laying hens for *Salmonella*. Most breeding flock monitoring was carried out at the hatchery via samples of meconium taken from hatched chicks during sexing. This was intended to identify transmission of *Salmonella* Enteritidis or *Salmonella* Typhimurium from parent flocks to progeny. This method suffered from both a lack of sensitivity in the case of low prevalence infection and a lack of specificity in that cross-contamination of chicks and meconium in the hatchery was a common occurrence, leading to the need to investigate multiple breeding flocks to identify which was the source of infection. At that time, confirmation was via post-mortem culture of pooled tissue samples from 59 birds per suspect flock. This only provides a potential detection threshold of 5% within-flock prevalence with 95% confidence, assuring a perfect test. The test was far from perfect as it was limited by the small size of the per-bird sample in the tissue pool, the “dilution effect” of pooling samples and the insensitive direct selenite broth enrichment method that was used. There were also strong suspicions of “confounding of results” by use of antibiotics before testing, and there was no way of identifying this available at that time. Most of the layer breeder industry also monitored flocks serologically, using a LPS-based indirect ELISA test to detect antibodies associated with exposure to *Salmonella* Enteritidis. Another test was available for *Salmonella* Typhimurium, but this was not so reliable and this serovar was never considered a significant problem in layer-breeder flocks.
- 5.2 Commercial layers were also monitored, but by means of pooled cloacal swabs. Cloacal swabbing was recognised to have low sensitivity but is favoured by the industry as it does relate to the birds themselves (Cooper et al., 1989). Confirmation of infection in either breeding flocks or commercial layers was followed by compulsory slaughter, with compensation which did not make up for losses in cash flow and failure to supply the specific market for eggs, so again there was a significant level of suspected manipulation, with reports of interference with the cloacal swabs being common. It was recognised that the legislation relating to commercial laying flocks was not working and in 1993 it was repealed, resulting in a lack of information regarding the *Salmonella* status of laying flocks at a time when the industry was becoming more intensive, with increased use of large deep pit cage systems and minimal cleaning and disinfection between flocks on large multiple age sites. This allowed the build-

up of large resident rodent populations, red mites and litter beetles and during this time *Salmonella* Enteritidis, which was originally acquired via infected replacement chicks before clearance of infection from breeding companies, become a permanent resident on most commercial scale cage layer units (Evans et al., 1999). A proportion of free-range and barn egg production units were also affected, but to a much lesser extent because farms and flocks were smaller, less likely to be multi-age and were usually subject to cleaning and disinfection between flocks. Persistence of infection in non-cage units, including breeding farms, was also normally associated with failure to control breeding rodent populations (Davies and Wray, 1995).

- 5.3 As part of the Lion Code scheme, the British Egg Industry Council introduced monitoring for *Salmonella* via a pooled sample of cloacal swabs and quarterly samples of 20 eggs; pooled for testing. This testing lacked sensitivity and of 100 infected flocks involved in research studies between 1999 and 2007, only 2 were detected by the monitoring programme that was in place. The Laid in Britain scheme used serological monitoring for detection of *Salmonella* in laying flocks, and although this should have been a more sensitive method, positive tests were not required to be reported to the competent authority and there was little indication of the efficiency of the test or its use to stimulate corrective action. Several Laid in Britain flocks with *Salmonella* Enteritidis were identified by the EU Baseline survey for *Salmonella* in laying hens that was carried out in 2003/4.
- 5.4 In the early- mid 1990s the occurrence of *Salmonella* Enteritidis in broiler breeder flocks was high and studies identified poor cleaning and disinfection and rodent control during the laying period as the main issues. Improvement in these aspects, plus the introduction of the inactivated *Salmonella* Enteritidis vaccine, Salenvac, improved the situation dramatically. On the layer breeder side, infection was less common as flocks and sites were smaller and more bio-secure, meaning that earlier infection that had originated high in the breeding pyramid could usually be cleared. Salenvac was occasionally used for replacement birds that were placed following an infected flock, but not on a routine basis. The main reason for this was that the industry wanted to continue to use serological monitoring, results of which did not have to be reported to Defra, to assess the *Salmonella* status of their flocks and to depopulate without official confirmation when necessary.
- 5.5 Between 1993 and 1997, cases of domestically acquired *Salmonella* Enteritidis continued to rise in the human population in GB and most outbreaks could be linked with domestically-produced eggs. Concerns about this situation amongst major retailers, and the egg industry led to a private serological survey of laying flocks carried out by the industry itself. This suggested that the majority of

flocks were infected at a time, in 1997, when human *Salmonella* Enteritidis cases were at their highest. A statement from the then minister of Agriculture stating that “most egg production is infected with *Salmonella*” was misinterpreted in the media resulting in a public reaction to the impression conveyed that “most eggs” were infected. As a response to retailer and consumer pressure and to minimise the associated large reduction in egg sales that occurred, the main industry quality assurance scheme, the Lion code, was launched to introduce the Salenvac vaccine, that had already been shown to be effective in broiler breeders, for laying hen flocks, as well as date stamping of eggs and improved farm hygiene and auditing standards. These changes were followed by a dramatic reduction in human cases and outbreaks, but it is unclear which element of the scheme; vaccination or date stamping to help avoid poor stock control at retail and catering, was the most effective. Owners of farms outside the Lion Code were unaffiliated or were members of the “Laid in Britain” scheme of UKEPRA. This scheme did not require vaccination, but rather recommended competitive exclusion as a cheaper and less laborious method to apply, but there is no evidence for the efficacy of this approach. Independent farms were free to do what they wished, and some medium sized farms supplying eggs locally were still implicated in *Salmonella* Enteritidis outbreaks due to egg consumption.

- 5.6 In the early 2000s, a *Salmonella* Typhimurium DT104 component was added to the Salenvac vaccine which was re-branded as Salenvac T. Observational research suggested that this was likely to be more effective against *Salmonella* Enteritidis than Salenvac and also provided additional protection against DT104, which was occurring at low prevalence in laying flocks at the time as a result of spread from the epidemic of infection from cattle, other food animal species and possibly people. Also at this time, the first live *Salmonella* Enteritidis vaccine; Tad Vac E, was launched in UK, having been developed and used in Germany for some years. A similar *Salmonella* Typhimurium vaccine was also available but that was little used initially, although it was the main vaccine used in Germany, where *Salmonella* vaccination of layers was compulsory, because it was cheaper than the specific *Salmonella* Enteritidis vaccine. Later, in response to the launch of Salenvac T, the live *Salmonella* Typhimurium vaccine, Tad Vac T, was offered free of charge to purchasers of VacE. This led to greater uptake, but with some complications since the licencing conditions forbade administration of both vaccines at the same time, but this was the only way that they could be administered effectively without one vaccine partially excluding the subsequent one. The vaccine was therefore administered contrary to data-sheet recommendations. Some large producers also began to use another live vaccine that was designed for protection against fowl typhoid, the Gallinarum 9R vaccine. This was cheaper than the other

vaccines but was required to be administered as a 2 dose course of subcutaneous injections; a very laborious procedure. The vaccine was therefore administered, contrary to data sheet requirements, as one oral dose and one intramuscular dose, the latter given when birds were transferred from rearing to laying sites. This vaccine was subsequently withdrawn after the occurrence of fowl typhoid on some farms where the vaccine had been used and an inability to exclude the possibility of reversion to virulence of some batches of vaccine (van Immerseel *et al.*, 2013).

- 5.7 When the live oral *Salmonella* vaccines were introduced in 2001, vaccination became more widespread amongst non-Lion Code flocks, but there was an increase in *Salmonella* which appeared to be associated with problems with vaccine administration through complex and unsuitable water systems in poultry houses. An education campaign was launched by the vaccine companies to promote correct administration, often requiring the installation of a metering device in the house to compensate for inadequate header tank capacity for mixing the vaccine.
- 5.8 A further live vaccine was introduced in 2003; Gallivac *Salmonella* Enteritidis. This was a live auxotrophic *Salmonella* Enteritidis PT4 vaccine which was more stable during administration but was also more persistent in birds and the environment, requiring introduction of a testing scheme to differentiate the live vaccine from field infections (Maurischat, *et al.*, 2015). Subsequently, a killed oil-adjuvanted *Salmonella* Enteritidis/*Salmonella* Typhimurium vaccine; Gallimune E & T, was introduced to UK to complement the Gallinarum *Salmonella* Enteritidis vaccine and provide additional protection against *Salmonella* Typhimurium in the face of the rise in monophasic *Salmonella* Typhimurium in pigs and the consequences of that in terms of cross-infection of poultry. The combination of 2 oral doses of Gallivac *Salmonella* Enteritidis plus one injection of Gallimune E & T became widely used, although again the single injectable dose was not in accordance with the data sheet. Experimental evidence suggests that combination of live oral and killed injectable vaccines provides better protection than either alone, but it has not been possible to confirm this in the field because of the economic consequences of finding a positive flock which might otherwise not have been detected. In 2013, 3 doses of Gallivac *Salmonella* Enteritidis was also licenced for protection against *Salmonella* Typhimurium and 3 doses was subsequently taken up as a standard requirement for Lion Code flocks. In 2014 a combined live vaccine for *S. Enteritidis* and *Salmonella* Typhimurium was launched; Avipro Duo, which overcomes the problems of co-administration of the two live vaccines.
- 5.9 Field observational research studies of *Salmonella* Enteritidis and Typhimurium positive flocks followed longitudinally the *Salmonella* status of several laying

farms through the initial introduction of vaccination and replacement of inactivated vaccine programmes with live vaccine between 2000 and 2008. Although the introduction of vaccination in these positive holdings was usually followed by some reduction in the flock prevalence and there was a suggestion of reduced contamination of eggs, all flocks remained infected with *Salmonella* Enteritidis despite vaccination (Davies and Breslin, 2004). The response of *Salmonella* Typhimurium to vaccination was better, but this serovar is also likely to generate a strong immune response after natural infection and to clear spontaneously (Carrique-Mas *et al.*, 2009). Deficiencies in cleaning and disinfection of poultry houses between flocks and control of pests such as flies, and particularly rodents, was the major issue, but it was not possible to persuade farmers to invest in better control measures until the introduction of the Salmonella National Control Programme in 2008, and particularly the threat of restrictions on the sale of fresh eggs from infected flocks that was planned for 2009. Success began in two farms where the farmer was persuaded to implement extremely intensive mouse baiting using peanut oil and pasta-based bait sachets. These two farms comprised 18 houses which were all persistently infected with *Salmonella* Enteritidis despite vaccination. Improved cleaning and disinfection including steam cleaning of cages instead of dry cleaning and disinfection using a formaldehyde- based disinfectant had been introduced but was undermined by immediate recontamination of housing by infected mice.

5.10 The elimination of infected mice with the intensive baiting programme was dramatic and surprisingly, in most houses, the *Salmonella* infection in birds rapidly cleared, even during the life of an infected flock. Propagation of these findings to industry through nationwide roadshows helped spread the message that this was possible and the success was soon replicated on a series of other farms, with almost all study farms totally clear of infection by 2009 (Davies and Carrique-Mas, 2010). In some cases farm managers who were not willing to carry out this intensive baiting properly had to be dismissed and replaced and there were a few failures in which lack of action led to closure of some farms, but continuation of some other infected premises, only to be detected as infected again years later by official NCP sampling, when the same strains of *Salmonella* were found.

5.11 One of the other main factors associated with clearance of *Salmonella* Enteritidis from laying farms was the requirement for replacement of conventional battery cages with enriched colony cage housing from January 2012. To install the new cage systems, houses had to be totally gutted, or replaced with new structures, which provided an opportunity to deal with resident rodent populations whilst the houses were empty with no feed available to distract the rodents from bait. Unfortunately, in a proportion of farms, the hollow walls and roofs had been colonised by mice which, after

insufficient baiting re-infected the new flocks that were placed in the new cages. A major advantage of the enriched cage system was that the deep manure pits, which previously harboured large populations of rodents and fly larvae on many farms, were eliminated as the new systems incorporated manure belts rather than manure pits. Each of the houses has multiple manure belts that feed a main belt that takes the manure out of the building to be stored in a central manure store on site. The disadvantages of this housing system are that the belt system is accessible for entry of rodents from the outside of the buildings so needs to be preventatively baited; a practice which is contrary to new HSE requirements for use of rodenticide biocides preventatively and outside building structures. It is also difficult to clean and disinfect colony cage houses as the cage stacks are often very tall and not readily accessible for washing. The cage “furniture”; involving internal perches, rubber matting and nest box flaps also complicates thorough cleaning, and many farms have reverted to dry cleaning only, with consequent increases in red mite populations which may increase the susceptibility of flocks to incursion by *Salmonella*.

- 5.12 The primary focus of control measures for *Salmonella* at the farm level is through the implementation of Regulation (EC) No. 2160/2003. This regulation provides a harmonised framework for determining the baseline prevalence of a specific zoonotic agent in animals at the farm level and procedures for setting a target to reduce this prevalence across the Community in a series of targeted National Control Programmes.
- 5.13 The survey mentioned above was designed to fit a set EC budget and this led to a maximum of seven samples to be taken per flock. In an attempt to increase the sensitivity of the testing it was agreed that two of the samples would be dust since *Salmonella* is easier to detect in dust than in faecal samples. The other 5 samples were either pairs of boot swabs for non-cage flocks or large pooled faecal samples for cage flocks. Flocks were not to be sampled within two weeks of antibiotic administration, as this may inhibit recovery of *Salmonella* from faecal samples in particular, and were to be taken towards the end of lay, when there is usually an increase in prevalence and number of organisms as the birds’ age, vaccinal “protection” wanes and vectors within a house increase. In all countries except the Republic of Ireland, where regular dust sampling has been used for routine monitoring, the prevalence of *Salmonella*, and in particular *Salmonella* Enteritidis, was significantly higher than previous National monitoring had suggested, even in the Nordic countries where *Salmonella* levels are very low. In Denmark, where both bacteriological and serological monitoring was carried out in laying flocks as part of a sensitive National programme, the detected prevalence in the survey was still higher and it was suggested that some owners of positive flocks had been guilty of deception when providing routine samples.

- 5.14 In the UK, the prevalence of *Salmonella* Enteritidis identified in the baseline survey was 5.8% and for *Salmonella* Typhimurium it was 1.8% (Snow *et al.*, 2010). These data, however, reflected the large number of very small non-cage flocks that were included in the survey, for which the prevalence would be expected to be low. The prevalence of *Salmonella* Enteritidis in large cage flock holdings was more than 50%, and was more than 25% in medium-sized (>30,000 birds) holdings. This means that a large proportion of eggs would have been produced on a holding with *Salmonella* infection present even in 2003/4. In addition to the limited sampling sensitivity, some flock owners were able to opt out of the survey and a substitute was found. Prior knowledge or suspicion of a *Salmonella* risk may have influenced the decision to opt out. Only one house per holding was sampled in the survey. Had all the houses been sampled the greater number of samples taken per holding would have increased the chance of obtaining positive samples (Carrique-Mas *et al.*, 2008a). The combined effort of these uncertainties means that the actual holding *Salmonella* prevalence would have been significantly higher than that found. The most valuable aspect of the survey was as a “wake up call” to the whole of Europe that all was not well with the egg industry and *Salmonella*.
- 5.15 The widely differing rates of *Salmonella* prevalence across Europe, and the recognised cost of dealing with clearance of infection on commercial laying farms, which in most countries involve multiple flock ages on the same site, so a clean break from housing birds is not possible, was problematic. It was therefore decided to set a sliding scale of target prevalence according to the national prevalence of *Salmonella* Enteritidis and *Salmonella* Typhimurium combined that was identified in the baseline survey (Carrique-Mas *et al.*, 2008b). The final flock prevalence to be achieved was set as 2%, but no criteria were set for the sizes of flocks so it would still be possible for a significant proportion of eggs to come from infected flocks if they were large and were not detected early in lay; a likely event (Van Hoorebeke *et al.*, 2009). In some countries, the initial high prevalence of infection meant that the flock prevalence in the years following the baseline survey remained relatively high, and this included some of the major egg exporting countries (EFSA CSR Reports 2008-2014). In most countries, infected flocks were allowed to remain in production but with eggs going for heat treatment. This means that infected birds remain on the farms and persistence of infection was therefore more likely (Dewaele *et al.*, 2012). In the Nordic countries no heat treatment option was allowed and flocks were slaughtered. This was also applied in GB since it has not been possible to support the costs of maintaining a flock on the reduced income from heat treated eggs, and many egg processing plants will not accept *Salmonella* positive eggs for processing, so it is uneconomic to continue in egg production.

In Northern Ireland, however, there is some capacity for processing infected eggs, including eggs from flocks outside that country.

- 5.16 It was originally envisaged that the baseline survey would be repeated after 3 years to evaluate progress, but funding constraints within the EU and considerations of how the identification of infected flocks could be dealt with under legislation when the selected flocks would be sampled with greater detection sensitivity than other flocks led to a decision not to repeat the survey but to rely instead on sampling carried out under National Control Programmes (NCPs) that were required to be introduced by all EU Member States. It was, however, considered that sampling with seven samples, as in the surveys, would be unjustifiably costly for the egg industry and eventually it was agreed that a single sample, repeated every 15 weeks during the laying period, starting when the birds are between 22 and 26 weeks of age would be acceptable, assuming that the cumulative sensitivity over the life of a laying flock would equal that of the one-off survey. This was based on a hypothetical calculation and in reality the difference in reported prevalence between the baseline survey and the first year of the NCPs in all countries suggested a marked reduction in sensitivity (EFSA CSR reports, 2008, 2009). An attempt to counter this was introduced within the NCPs in that holdings with more than 1000 birds were to be officially tested and a dust sample would be taken in addition to the boot swab or pooled faeces sample, with these samples being tested in an official laboratory rather than in a private one in which the operator samples were tested. In those countries where data are available there has been up to 10-fold greater prevalence detected by official sampling and testing than by operator sampling and testing (Arnold *et al.*, 2014). Only one house per holding is sampled once a year, so on larger holdings so the official sampling represents a small fraction of the overall testing. It is not certain what contributes to this discrepancy, but the thoroughness with which a representative pooled sample is taken, its timely and careful transit to the laboratory and the specific variables within the implementation of testing methods may all play a part (Gosling *et al.*, 2014). In the UK, it was found that detection can be affected by the temperature of transit of the sample, especially for boot swabs, the amount of the BPW which should totally submerge the sample during pre-enrichment, the specific brand of modified semi-solid Rappaport-Vassiliadis Medium (MSRV) and its reconstitution, control of incubation temperature, the plating method used and selection of suspect colonies for confirmation (Gosling and Davies, 2015). The most common issue is overgrowth of the *Salmonella* by competing bacteria in the sample. This is why dust, as used in the Lion code programme for pullet flocks before entering the laying phase, is a more effective sample for detection of flock infection than faeces, as the proportion of competing *Enterobacteriaceae* is hugely reduced compared to faeces, even though total

bacterial counts may be equivalent, and *Salmonella* survives better than most competing organisms under dry conditions (Martelli *et al.*, 2014).

- 5.17 The large financial penalty of restrictions on the sale of eggs from infected flocks has been a major incentive to improve but also has led to the introduction of various levels of confirmatory sampling in case there has been a false-positive result as a consequence of cross-contamination. In some countries where there is a very low level of *Salmonella*, such confirmatory testing has never been allowed as the risk of negating a genuine positive result is considered too high. Confirmatory testing following an operator positive result is allowed in the UK. An additional layer of optional voluntary confirmatory testing is also permitted. This allows producers to purchase a test of 4,000 eggs or 300 birds (or the same seven environmental samples that were used in the baseline survey), to further test the flock, even if there has been more than one positive test previously. Interference with these tests is possible through the use of antibiotics before the samples are taken. This is known to have happened during previous *Salmonella* control programmes in breeding flocks and as a precaution EC introduced a regulatory requirement that antibiotics should not be used to control subclinical *Salmonella* carriage in chicken flocks and recommended that checks be carried out for antibiotic use before testing, particularly for confirmatory samples. The lack of a suitable, affordable test for detecting all relevant antibiotics has been a significant impediment to this check, since standard bacteriological antibiotic residue tests on liver and kidney can only detect antibiotics that are systemically absorbed from the intestine but agents such as colistin, a commonly used antibiotic in poultry, cannot be detected after oral administration (Roudauf, 1989).
- 5.18 Similar considerations apply to the testing of boot swabs, anecdotal reports information from small smaller UK farms by APHA field staff that lime may sometimes be applied to litter before confirmatory sampling with boot swabs. Unlike antibiotics, this is not illegal, but is likely to significantly reduce the chance of detecting *Salmonella*. High levels of organic acids given in the feed and drinking water of caged flocks can have a similar effect (Van Immerseel *et al.*, 2006). Such considerations, plus the ongoing occurrence of *Salmenlla* Enteritidis due to eggs and egg products in the EU, and the fact that *Salmonella* Enteritidis is still by far the most common serovar occurring in people in the UK and EU suggests that although there may have been major improvements at farm level, there is still a risk of failure to detect infection, particularly in flocks in egg-exporting countries where there is a high average temperature and/or relatively uncontrolled use of antibiotics in poultry (Mølbak *et al.*, 2014, de Knegt *et al.*, 2015).

- 5.19 In the UK there has been no significant issue regarding layer rearing flocks in recent years, and control of *Salmonella* in such flocks is much easier since the occupation time is shorter and housing is more amenable to pest control and effective cleaning and disinfection. The fortnightly or three weekly more sensitive monitoring carried out at breeder level as required by EU legislation is likely to detect *Salmonella* Enteritidis if it is present so chicks are unlikely to be infected at hatch. Occasional cases of *Salmonella* Typhimurium have been identified in breeding flocks, largely associated with introduction of monophasic *Salmonella* Typhimurium, and in one case day old layer chicks became infected with *Salmonella* Typhimurium DT99, a host-adapted pigeon type, after contamination of a hatchery, but these are very rare events and no *Salmonella* Enteritidis has been found in layer breeding flocks in UK for nearly 20 years. The additional *Salmonella* monitoring, which is mandatory under the Lion Code scheme carried out in larger hatcheries would also be likely to detect *Salmonella* Enteritidis if it was present, because of its strong vertical transmission characteristics (Davies *et al.*, 1997).
- 5.20 The two main farm assurance schemes have focused particularly on *Salmonella* control in order to protect egg sales. As well as the vaccination and monitoring initiatives described above, the Lion Code Scheme⁴ sets hygiene standards for all stages of egg production and stipulates a maximum storage temperature for eggs on farms of 20°C, after removal from laying houses, which aim to be maintained at 21°C, but which may experience significantly higher temperatures on the occasional hot days that occur in the UK. A seasonal effect of high temperature on increased *Salmonella* occurrence in laying birds within a flock is also described. The schemes employ independent auditors from a UKAS accredited assurance scheme certification body who visit all holdings once a year, which is combined with the official sampling of flocks. The standard of sampling has never been physically audited and although the isolation rate of regulated *Salmonella* serovars has been lower than non-scheme farms since the introduction of the NCP, the populations of birds are not equivalent. The standard of auditing of farm biosecurity standards is, however, sometimes open to question as farms that have proved to be positive have had significant rodent problems that have not been identified by auditor visits.
- 5.21 The caveats above very much relate to detection of infection within an individual flock however, taking context of a National program where there is analysis of hundreds of thousands of samples and in conjunction with the human data considered elsewhere in the report, it is clear from both farm and

⁴ <http://www.britisheggindustrycouncil.co.uk/british-lion-code-of-practice/>

human data that British table eggs are no longer a relevant source of *Salmonella* infection, and that the UK chicken industry has the lowest rate of regulated *Salmonella* serovars of any major poultry producing nation. UK eggs produced under comprehensive schemes mentioned above, in particular, present a minimal risk to human health.

***Salmonella* National Control programmes at primary production**

- 5.22 From the late 1980s there have been statutory *Salmonella* control programmes for certain sectors of the poultry industry in the UK. These controls have been amended over the years but since 1992 up until 2007 they have been based on the requirements of Zoonoses Directive (EC) No 92/117. This piece of legislation required Member States to monitor the trends and sources of various zoonotic agents in animals, feed, food and man, analyse them and report the findings to the Commission. In addition it required Member States to take certain action on breeding flocks of domestic fowl to control *Salmonella* Enteritidis and *Salmonella* Typhimurium. The action taken on breeding flocks of domestic fowl (*Gallus gallus*) was to monitor each breeding flock, and if *Salmonella* Enteritidis or *Salmonella* Typhimurium was confirmed to be present in the breeding flock, the breeding flock was slaughtered. The monitoring of the breeding flock took place at hatcheries with follow up confirmation in the birds on the farm.
- 5.23 A review of how the Directive (EC) 92/117 which was carried out in the late 1990s by the Scientific Committee on Veterinary Measures relating to Public Health, and in its opinion published in April 2000 it was considered that the measures in place at that time to control foodborne zoonotic infections were insufficient, and it went on to propose other risk management options. As a result in 2003 Member States agreed that the monitoring of specified zoonotic agents should be expanded, and harmonised where beneficial in a new Directive (EC) No 2003/99, and that the risk management measures required to control zoonotic infections should be extended in a new Regulation (EC) No 2160/2003 on the control of *Salmonella* and other specified foodborne zoonotic agents.
- 5.24 Therefore, currently the primary focus of control measures for *Salmonella* at the farm level is through the implementation of Regulation (EC) No. 2160/2003. This regulation provides a harmonised framework for determining the baseline prevalence of a specific zoonotic agent in animals at the farm level and procedures for setting a target to reduce this prevalence across the Community in a series of targeted National Control Programmes. *Salmonella* reduction targets have been set and programmes implemented in all Member States in the specified poultry species: breeding chickens, laying flocks of chickens producing eggs for human consumption, broilers, breeding turkeys and

fattening turkeys. Member States are required to implement programmes, which cover the whole of the food chain and monitor the progress towards achieving the reduction target, and to take action to achieve the reduction, along with other specified measures to protect public health.

- 5.25 Overall, the principles are a step-wise approach to *Salmonella* control along the whole food chain and the application of at least the minimum harmonised monitoring requirements and risk management measures with specific emphasis on the *Salmonella* serovars of most public health importance. Implementing regulations prohibit the use of antimicrobials to control *Salmonella* except in specific defined circumstances where the welfare of the birds is compromised by clinical disease or for conservation of rare breeding stock. Vaccines may be used for control as long as the vaccine used is authorised in the Member State and a method is available to differentiate vaccine from field/wild strain *Salmonella*. Additionally, in countries where the prevalence of *Salmonella* Enteritidis and *Salmonella* Typhimurium was greater than 10%, vaccination is required to be used unless a specific derogation is obtained. The EU Commission provides co-financing (at a maximum of 50% of total expenditure in the member State) for the measures required by the programmes including the payment of compensation for mandatory slaughter, vaccination, official sampling and laboratory testing and post cleaning and disinfection sampling, to facilitate effective implementation of the programmes in each Member State.
- 5.26 The *Salmonella* NCP in breeding chicken flocks was implemented in January 2007. For the breeding chicken sector, the target was based on the monitoring results of the previous control programme and the target includes five serovars that were the most common serovars in laboratory confirmed cases in humans at the time: *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Salmonella* Hadar, *Salmonella* Infantis, and *Salmonella* Virchow. The reduction target for breeder flocks is for less than 1% of flocks to be infected with these five serovars annually. The programme requires operators to take samples from each breeding flock every two or three weeks during the laying stage, and for these to be verified by official sampling on two or three occasions during the life of the flock. In addition, during the rearing phase, breeder chicks have to be sampled at day-old, four weeks of age, and around two weeks before they come into lay or before moving to the laying accommodation.
- 5.27 If *Salmonella* Enteritidis or *Salmonella* Typhimurium is confirmed in a flock, it is slaughtered, and any hatching eggs present in the system since infection was confirmed are removed and destroyed. The aim of this mandatory requirement in all Member States is to prevent the vertical transmission of *Salmonella* Enteritidis and/or *Salmonella* Typhimurium from the breeding hen through the

hatching egg to the day old chick. Provided this operated effectively it was expected that day old chicks would be free of *Salmonella* Enteritidis and *Salmonella* Typhimurium when they were placed on farms. If *Salmonella* Hadar, *Salmonella* Infantis, or *Salmonella* Virchow is confirmed in the breeding flock, the operator is required to draw up a plan to reduce it in collaboration with the operator's veterinarian, and government officials.

- 5.28 The *Salmonella* NCP in laying chicken flocks was implemented in February 2008. The reduction target was based on the results of the EU – wide baseline survey carried out in 2004-2005 (EFSA 2007) which indicated a wide range of *Salmonella* prevalence in the Member States and so was set as a percentage reduction of infected flocks based on the baseline prevalence up to a maximum of 2% of flocks detected positive for *Salmonella* Enteritidis and *Salmonella* Typhimurium annually. Owners of layer flocks are required to sample their flocks during the laying phase every 15 weeks, starting when the flock is 22 to 26 weeks of age. Official verification samples are required from one flock on all premises with more than 1000 birds. In addition, the pullets are sampled when they are day old, and again around 2 weeks before they come into lay or are moved to the laying accommodation.
- 5.29 If *Salmonella* Enteritidis or *Salmonella* Typhimurium is confirmed in a flock, egg marketing restrictions are applied and the eggs may not be marketed for human consumption unless heat treated to eliminate *Salmonella* contamination prior to consumption (ie may not be marketed as fresh Class A table eggs). The eggs are therefore only to be marketed as Class B eggs and must be marked individually to designate the Class B status. This requirement applies for the remainder of the productive life of the flock. If a flock is identified as the source of a foodborne outbreak of salmonellosis caused by any *Salmonella* serovar (i.e not just the target serovars *Salmonella* Enteritidis and *Salmonella* Typhimurium), the egg marketing restrictions also apply.

Research on control of *Salmonella* in the duck egg industry

- 5.30 Duck eggs have traditionally been associated with *Salmonella* risk, but this has probably been overlooked by modern consumers who have less knowledge and involvement with primary food production than previous generations. Following the outbreak of *Salmonella* Typhimurium DT8 associated with duck eggs in 2010, a series of investigatory research visits was launched to duck-farming premises where *Salmonella* Typhimurium or *Salmonella* Enteritidis had been identified by voluntary surveillance or as a result of trace-back investigations following human cases where the source of eggs was known. Epidemiological tracings had suggested that one duck breeding company was likely to be associated with cases in England, Northern Ireland and the Republic of Ireland. There had been considerable expansion of duck egg

production, breeding of laying ducks and onward supply of birds for use within the company and to supply other companies and small flocks. The original stock for the farm came from France, where *Salmonella* Typhimurium DT8 has been reported frequently, and also from another breeding company in the UK, which used to have this PT as an endemic strain, but subsequently cleared the infection by means of antibiotic treatment of hatching eggs; a procedure which is no longer legal for chickens and turkeys within the EU except for exceptional cases in which irreplaceable genetic stock would be lost. The duck industry is, however, outside this legal framework to some extent, so such practices, as well as routine medication to suppress *Salmonella* in breeding flocks can still be used. None of the flocks involved in the egg outbreak were subject to such medication and whole genome sequencing suggested that the human outbreak strains were more closely related to French isolates than pre-existing UK isolates.

- 5.31 In response to the *Salmonella* Typhimurium DT8 outbreak in people, and the epidemiological investigation carried out by PHE, as well as isolation of the strain from a dead-in-a shell embryo sampled in the hatchery, APHA launched a Defra-funded field investigation of all duck holdings where DT8 or *Salmonella* Enteritidis PT9b had been reported, as well as premises linked with these. Intensive sampling was carried out to define the distribution of infection/contamination on the farms and associated hatcheries and advice was provided on how best to deal with the infection.
- 5.32 A major complication of *Salmonella* control efforts on duck farms is the practice of breeding replacement breeding birds from existing stock, thus guaranteeing continuous recycling of *Salmonella* if it is present. Another complication is the multi-age production, sometimes with birds of different ages kept within the same house, making all-in, all out stocking programmes very difficult to implement. On the DT8 index case farm, there were also problems with mice inhabiting hollow block walls in several of the buildings, the products and concentrations used for disinfection of houses after de-stocking, and the within-site biosecurity arrangements; with no boot changes between houses and birds being walked between houses when moved between the different rearing and production stages. In the hatchery there were problems of residential contaminated dust in incubator ventilation ducting and fan drive mechanisms. Eggs were often dirty when collected but were washed or bleached using an effective wash machine or sodium hypochlorite soak tank. Within the project, eggs were collected and tested from a range of *Salmonella*-positive flocks and despite a high level of infection in most flocks, eggs were rarely test-positive and only one egg with external contamination was identified. Interventions included upgrading disinfection of duck housing using a very effective aldehyde-based product that is still active in the presence of organic matter,

upgrading the hatchery cleaning using specially designed brushes for cleaning inaccessible areas within hatcher cabinets and upgrading hatchery disinfection, rodent control involving baiting of wall cavities with second generation anticoagulant bait via blocks, introducing a double boot dip and boot change on entry to each house and upgrading the vaccination programme to add live vaccine to the existing inactivated vaccine program. Of particular importance was administration of live vaccine to day-old ducklings by coarse spray at the hatchery in an attempt to get vaccine into birds at the earliest possible point before spread of infection. Spray administration also results in an enhanced immune response compared with administration in the drinking water (Atterbury *et al.*, 2010), which is what is done normally. Since all these interventions are applied to birds that are already infected and these remain on site for up to two years, progress on eliminating infection is necessarily gradual, and depends on sequentially reducing the prevalence and thereby increasing the proportion of birds that receive vaccine before exposure to infection to the point where recycling infection is no longer biologically sustainable (Kim and Johnstone, 2011). In this case the combination of measures that were applied resulted in a gradual reduction in infection, followed by a sudden total clearance of *Salmonella* throughout the company as infected rearing flocks were replaced by birds with good vaccinal protection.

- 5.33 The inactivated vaccine that was already in use within the company is likely to have provided significant protection against extra-intestinal infection and therefore infection of table eggs, and eggs from this site were never implicated in the outbreak. Day old birds intended for table egg production were however supplied to a range of other producers, including in Northern Ireland and the Republic of Ireland, and some of these were the flocks that were identified in trace back exercises. These flocks were typically not vaccinated against *Salmonella*. Additionally, spent-ducks at the end of their productive life with the company were sometimes sold on to other small scale egg producers, or kept for a further cycle of lay. Although these birds would have had a primary vaccination course, a booster dose would be needed to maintain a reasonable level of protection for a second laying period, and if forced moulting was applied to induce a more productive second laying period, the stress of this would be likely to increase the level of *Salmonella* in infected birds (Berry, 2003). Moulting appears to be relatively common in the duck breeding and table egg production sectors.
- 5.34 Similar but targeted approaches were applied to other infected duck farms (as well as one quail egg producer) that had *Salmonella* infection and all who followed the advice were able to eliminate infection by the end of the study. The project also involved meat ducks, but none of these farms had DT8. Control of *Salmonella* in meat duck production is much more difficult as no vaccine exists

for the range of serovars that are commonly found and practices such as daily entry of straw chopper vehicles to houses and very short turnaround times make effective control extremely difficult, even though *Salmonella* Enteritidis and *Salmonella* Typhimurium can be reduced in a similar way by vaccination and improved control measures in meat duck breeding flocks.

Conclusions

- 5.35 Introduction of vaccination for *Salmonella* Enteritidis in the egg industry was associated with a large reduction in human cases.
- 5.36 Although *Salmonella* Enteritidis was not uncommon on large laying farms prior to the introduction of NCPs, the combination of vaccination, rodent control and improved farm hygiene standards, together with the removal of traditional battery cage systems, resulted in virtual eradication of infection in British laying flocks by 2009, and the prevalence further reduced since that time.
- 5.37 Taking context of a National program where there is analysis of hundreds of thousands of samples and in conjunction with the human data considered elsewhere in the report, it is clear from both farm and human data that British table eggs are no longer a relevant source of *Salmonella* infection, and that the UK chicken industry has the lowest rate of regulated *Salmonella* serovars of any major poultry producing nation. UK eggs produced under comprehensive schemes mentioned above, in particular, present a minimal risk to human health.
- 5.38 The lack of a harmonised control programme for ducks across the EU means that routine monitoring of flocks at all levels of production is much less effective than in the chicken and turkey sector, and many producers do not do any routine testing, or use methods that are inherently insensitive. The introduction of the Duck Assurance Scheme by the British Poultry Council was an attempt to raise standards closer to those applied in chicken and turkey flocks, but there is still some way to go to persuade all producers to fully participate in the absence of a statutory control programme.
- 5.39 Research has shown that similar approaches to those used in the layer industry can help eliminate *Salmonella* infection from duck breeding and egg production farms

(b) Other interventions

- 5.40 Hygiene controls on eggs both at primary production and further down the food chain relevant to limiting/mitigating the risk of *Salmonella* contamination can be divided broadly into the on farm controls as detailed above, good hygiene practice based on guidance, Codes of practice and specific measures required

by farm assurance schemes at various stages of the egg production chain and the statutory requirements of Regulation 2160/2003, the Food Hygiene legislation and egg marketing regulations.

Provision of Guidance:

Guidance to Food Business Operators

5.41 Articles 7-9 of Regulation (EC) No. 853/2004 on Hygiene of Foodstuffs detail the requirement for development of guides for good hygiene practice. COPA-COGECA (the Committee of Professional Agricultural Organisations in the European Union and the General Confederation of Agricultural Co-operatives in the European Union) and EUWEP (the representative body in the European Union for egg packers, egg traders and egg processors, and poultry and game) have published such guidance for the laying chicken sector: The *Community Guide for Good Hygiene Practices in Pullet Rearing and Egg Laying Flocks* (http://ec.europa.eu/food/food/biosafety/salmonella/docs/community_guide_layers_hygiene_practice_pullet_egg_en.pdf). The guide covers production and collection of eggs at the farm – risk management, biosecurity, C&D etc and, although it has no formal legal status, it complements other Codes of Practice that are in operation in the Member States and OIE recommendations. There are also various other Guides and Codes of practice that have been produced by Defra/APHA in various formats to provide information and guidance to the UK laying chicken sector on *Salmonella* control (add refs)

Guidance to consumers

5.42 The Government advises consumers on ways to avoid the risk of food poisoning, these include buying eggs from a reputable suppliers, and then store, handle and cook eggs properly. This advice especially applies to people in vulnerable groups, including the very young, the unwell, pregnant women and elderly people.

<http://www.nhs.uk/Livewell/Goodfood/Pages/eggs-nutrition.aspx>

Other statutory requirements

5.43 A requirement for 'best before date' to be applicable to shell eggs marketed as Class A eggs is set in Regulation (EC) No. 589/2008 as 28 days from laying. This period of time is based on egg quality criteria rather than food safety considerations although in combination with other factors including inadequate storage could have a potential food safety impact. The Hygiene Regulations, specifically Regulation 853/2004, specifies a 'sell by date' of 21 days – i.e. table eggs must be placed on the market within a maximum of 21 days after lay. This requirement aims to provide a reasonable and harmonised table egg shelf life for consumers. Although neither of these requirements relate directly to food

safety considerations specifically in relation to mitigating the risk of *Salmonella* infection transmission via eggs, they are considered to have an impact on reducing risk due to the inherent physical and chemical defence mechanisms against microbial contamination and growth that fresh chicken eggs have (Humphrey, 1994). According to the recent EFSA Opinion already mentioned (EFSA 2014), extending either one or both of these requirements would result in an increased relative risk of *S. Enteritidis* infection.

- 5.44 Food Hygiene legislation, specifically Regulation 852/2004 and Regulation (EC) 853/2004 also define specific hygiene requirements for production of eggs and egg products, covering primary production, collection, storage, transport, packing, record keeping etc.
- 5.45 **The microbiological criteria Regulation (EC) No. 2073/2005 egg products:** where the manufacturing process or the composition of the product will not eliminate *Salmonella* risk are subject to testing against defined microbiological criteria – a food safety criterion for *Salmonella* and a process hygiene criterion for Enterobacteriaceae. The food safety criteria is for absence of *Salmonella* in 25 g or ml (sampling protocol $n=5$ and $c=0$) but it should be noted that the testing frequencies are not laid down in EU legislation as testing protocols are should be based on HACCP-based procedures and good manufacturing principles.
- 5.46 Robust, evidence based methods for the validation of the performance of heat-processing treatments are probably required because current methods may overestimate the microbial load reduction achieved, with industrial procedures for heat treatment of egg products.
- 5.47 Egg washing: According to Regulation (EC) 589/2008, washing of Class A table eggs (produced by hens of the species *Gallus gallus*) is generally not permitted. Washed table eggs may only be marketed in the MSs in which an authorisation for such practice has been issued. This practice is not permitted in the UK. However, egg washing before processing is permitted in the EU (Regulation (EC) No 853/2004, Regulation (EC) No 589/2008), provided that the eggs are dry before they are broken. An evaluation of advantages and disadvantages of egg washing was carried out by EFSA in 2005 (EFSA 2005) which indicated that if the washing process was well done, there were clear advantages to egg washing because of the reduced microbial load, although this needed to be considered in the light of particular washing systems. It was concluded that poor practices increase the risk and the greatest risk in relation to egg washing was considered to be penetration of the egg by *Salmonella* species. Most notably, it was concluded that, in countries where the *Salmonella* prevalence in layers is very low, the risk of egg washing will also be lower. Currently, for economic and technical reasons (including the technical challenges in the

process) as well as to avoid possible problems associated with ineffective washing, egg washing is not a current practice in European egg-processing plants.

5.48 **Controls on Trade:** As part of the general requirement for imports of poultry and poultry products, third countries must comply with the same public health risk reduction requirements as those in place in the EU. Therefore, for third countries wishing to export eggs to the EU, Article 10 of Regulation (EC) No. 2160/2003 requires that the country must implement equivalent measures for the control of zoonoses and have in place a *Salmonella* National Control Programme approved by the EU Commission. The conditions for the importation of eggs and egg products are laid down in Commission [Regulation \(EC\) No 798/2008](#) and the list of third countries that comply with this requirement and therefore can export Class A fresh table eggs into the EU is included in the table in Annex I, Part 1 of Regulation 798/2008. Countries that do not have a programme are eligible only to export Class B eggs to the EU. Class B eggs have to be marked so that they can be distinguished from, and cannot be diverted and sold as Class A eggs. The mark required is described in Article 10 of Regulation (EC) No. 589/2008.

5.49 All imports of Class A eggs must come with the veterinary certificate for eggs, the Class of the eggs must be clearly marked and the eggs must be certified to originate from flocks that comply with the requirements of Regulation 2160/2003 and Regulation 1168/2006 (as amended by Regulation (EC) No. 517/2011). Class B eggs must also come with veterinary certificate for eggs, must be recorded as Class B eggs and, additionally, all eggs must be individually marked. There is no derogation from this marking requirement for eggs delivered directly to the food industry if the third country does not have the correct listing in Regulation 798/2008 indicating an equivalent *Salmonella* programme is in place in the third country (as specified in Article 11 of Regulation (EC) 598/2008).

Recommendation

5.50 Robust, evidence based methods for the validation of the performance of heat-processing treatments are probably required because current methods may overestimate the microbial load reduction achieved, with industrial procedures for heat treatment of egg products.

(c) Scientific robustness of anti-*Salmonella* interventions

Background

5.51 The purpose of this chapter is to try determine the robustness of the many anti-*Salmonella* measures applied, principally against *Salmonella* Enteritidis, in UK

hens' egg production in the last ~30 years, mainly under the Lion Code, and discussed in chapters 7a and 7b. The potential accuracy of the data showing changes in *Salmonella* Enteritidis infections in humans and chickens will also be discussed, as will reasons for possible error and variation in reported data in the UK and elsewhere. It will be difficult to compare and determine the robustness of individual *Salmonella* control measures such as improved biosecurity and better rodent control against, for example, vaccination, date-stamping and the introduction of a maximum temperature for on-farm storage of eggs. Many interventions were applied simultaneously, and there have been no rigorous scientific assessments of their impact, in isolation or combination.

5.52 The EFSA document “Scientific Opinion on the public health risks of table eggs due to deterioration and development of pathogens” attempted to evaluate the impact of individual EU member states *Salmonella* control programmes on public health, the incidence of human salmonellosis cases caused by *Salmonella* Enteritidis, the numbers of *Salmonella* foodborne outbreaks caused by eggs and egg products, and the prevalence of *Salmonella* Enteritidis in laying hen flocks, as did ECDC (2013). At the EU level, the proportion of *Salmonella* Enteritidis-infected laying hen flocks during the production period decreased steadily from 3.9% in 2007 (19 reporting Member States) to 1.3% in 2011 (27 reporting Member States). Between 2007 to 2011, the proportion of *Salmonella*-positive table eggs decreased from 0.8 % in 2007 (16 reporting Member States) to 0.1 % in 2011 (13 reporting MSs), and a 60.5 % reduction in the notification rate of human *Salmonella* Enteritidis cases per 100 000 population was observed (from 21.0 to 8.3). There was also a corresponding 42.3% reduction in the number of *Salmonella* foodborne outbreaks caused by eggs and egg products reported in the EU from 2007 to 2011 (a decrease from 248 to 143 outbreaks). In the annual report on European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks 2014, EFSA reports that in 2014, this statistically significant decreasing trend of salmonellosis continued. *Salmonella* was rarely found in table eggs, at levels of 0.3% (single samples) or 1.0% (batch samples) In 2014 in total, 0.4% of the 13,394 tested table egg units were found to be *Salmonella*-positive (0.3% of single samples and 1.0% of batches). Most of the tested units were reported by Germany (53%) and Poland (23%). In 2013, a total of 23,441 units of table eggs were reported to have been tested, 0.1% of which were *Salmonella*-positive. Generally, the proportion of positive units has been very low for the last couple of years. However, only few MS report data, reporting MS change between years and consideration of what constitutes a batch or single sample varies considerably in terms of weight (25–500 g) and content (white, yolk or whole eggs) among the

MS. For egg products, none of 17 batches were found to be positive for *Salmonella*, whereas three out of 636 (0.5%) single samples tested positive.

- 5.53 The above reports note that the *Salmonella* control programmes now in place in Member States are intended to have an impact on the whole food chain from farm to fork, and that a reduction in *Salmonella* at the farm level is expected to reduce the risk of salmonellosis in humans. In addition, other control measures along the food chain during processing, distribution, retail and food preparation are also important in reducing the risk. The above results indicate that the reduction of *Salmonella* Enteritidis in laying hen flocks and of *Salmonella* in table eggs are likely to have contributed to the decline of *Salmonella* Enteritidis cases in humans.

Production Control measures

- 5.54 Numerous methods have been explored to control *Salmonella* contamination throughout the egg production process. One of the basic methods is routine cleaning and disinfection between flocks. However, the effectiveness of these cleaning routines can be highly variable. Wales *et al.* (2006) investigated 12 *Salmonella*-contaminated caged layer houses after cleaning and disinfection, and found that none were completely *Salmonella*-free. Another study by Davies and Breslin (2003) compared the effectiveness of cleaning and disinfection in free range, barn and cage layer housing, and observed a decrease in *Salmonella* contamination in free range housing although the soil remained contaminated. However, in the barn and cage housing significant contamination remained on the surfaces of buildings and equipment. Anecdotally, it has also been suggested that there may be reduction in contamination as a result of modern farming methods. For example, modern barn systems disposing of faecal material via manure belts would have lower contamination than older barn systems which would allow faecal material to pool until restocking, and colony cage houses that use manure belts rather than the deep pit systems used previously are also cleaner and less attractive to rodents. Housing systems will be discussed in more detail later in this chapter.

Vaccination:

- 5.55 In a review paper O'Brien (2013) concluded that vaccination of laying hens in the UK, which began in 1997, made a significant contribution to the reduction in human *Salmonella* Enteritidis cases, observed during the late 1990s. Previous interventions such as attempts at improved external biosecurity/eradication of infection from breeding flocks and test/slaughter policies had not achieved reductions. Such data suggest that vaccination of laying hens was the most

important and successful intervention for prevention of human infection, even though vaccinated flocks often remained infected. There are considerable data showing that flock infection and egg contamination with *Salmonella* Enteritidis has declined, not only in the UK and in the EU, but also in many other parts of the world. We will examine the robustness of these data, and discuss the impact of other interventions such as changes in bird housing, brought about by EU regulations and the evolution of *Salmonella* Enteritidis into over 200 PTs, and possible impact on the efficacy of PT4 vaccines. Contamination of eggs with *Salmonella* is a complex issue that is influenced by many variables, making it difficult to implement appropriate management strategies (Whiley and Ross 2015) or measure their impact.

5.56 Vaccination of hens has had varying success against *Salmonella* infection, depending on the vaccine and the *Salmonella* serovar. Berghaus *et al.* (2011) demonstrated that a vaccine containing killed *Salmonella* Typhimurium, *Salmonella* Enteritidis, and *Salmonella* Kentucky increased the immunity of the hens and their progeny against those particular serotypes, but did not decrease the incidence of *Salmonella* in environmental samples taken from the housing. Arnold *et al.* (2014) found that vaccination did not influence the proportion of hens shedding *Salmonella* Enteritidis and *Salmonella* Typhimurium, but did significantly decrease the incidence of both serovars on eggshells compared to the non-vaccinated hens. Both live and inactivated vaccines are available for the control of *Salmonella* Enteritidis and/or *Salmonella* Typhimurium in the EU. In the UK most vaccines used are live attenuated ones. The protection offered by vaccination is often not complete or sustained, although the likelihood of infection of eggs is reduced (Davies and Breslin, 2003). Detection of infected flocks may also be reduced as a result of reduction of the within-flock prevalence (Berghaus *et al.*, 2011) and number of organisms shed in faeces (Van Immerseel *et al.*, 2004; Gantois *et al.*, 2006; Inoue *et al.*, 2008). Waning of vaccinal protection may be involved in the rise in excretion towards the end of lay.

5.57 The immune response of chickens against *Salmonella* infection is both innate and acquired. When *Salmonella* first reaches the intestine, it invades the intestinal epithelium, rapidly attracting immune cells (such as polymorphonuclear leucocytes and macrophages) to the site (Van Immerseel *et al.*, 2005). More than 90 % of the *Salmonella* cells that invade beyond the intestinal mucosa are destroyed by these phagocytic cells. *Salmonella* has adapted to grow inside host macrophages, which become sites of bacterial multiplication and vehicles for systemic distribution of the bacteria via the lymphatic and blood circulatory systems to other organs (Uzzau *et al.*, 2000), most significantly the reproductive tract. Non-paratyphoid infections in

chickens are largely restricted to the intestinal lumen and evoke an acquired immune response, which mainly involves the production of immunoglobulin (Ig) A (as it can be secreted across intestinal epithelia and into the lumen). Clearance of primary *Salmonella* Enteritidis and *Salmonella* Typhimurium infection is dependent on age of the chicken and host genetics (Beal and Smith, 2007). Cell-mediated immunity plays a more important role than the humoral response in protection and against *Salmonella* infection (Van Immerseel *et al.*, 2005). Vaccination of laying hens against *Salmonella* Enteritidis and *Salmonella* Typhimurium has been shown to not only confer protection against infection in birds but to also decrease the level of on-farm contamination (Van Immerseel *et al.*, 2005).

- 5.58 As *Salmonella* Enteritidis and *Salmonella* Typhimurium are considered to be the most important serovars for public health in Europe, current commercially available live and inactivated *Salmonella* vaccines for poultry are intended for use against one or both of these serovars. In some European countries (Austria, Belgium, The Czech Republic, Germany and Hungary) vaccination of laying flocks is compulsory, in others vaccination is permitted and recommended (Bulgaria, Belgium, Cyprus, Estonia, France, Greece, Italy, Latvia, Lithuania, The Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain and the UK) while in others it is banned (Denmark, Finland, Sweden and Ireland) (Galiş *et al.*, 2013).
- 5.59 Vaccination is used to prevent systemic infection (and localization in the reproductive tract) and to reduce faecal shedding (and consequently carcass and/or egg contamination). Vaccination is regarded only as an additional measure to increase the resistance of chicks to *Salmonella*, especially if the flock prevalence is high, but it is a very important one. 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 faecal shedding, ovarian transmission, and the within-flock prevalence, thereby reducing contamination of table eggs and the environment. Most importantly, the use of vaccination against *Salmonella* Enteritidis and *Salmonella* Typhimurium seems to lower internal-egg contamination levels, thereby most directly contributing to improvements to public health (Davies and Breslin, 2004; Gantois *et al.*, 2006). In the EU-wide baseline study conducted in 2004-2005, vaccination of laying flocks was found to decrease the risk of *Salmonella* Enteritidis compared with unvaccinated flocks. Vaccination was demonstrated to be particularly effective in Member States with high farm prevalence (more than 15 %) of *Salmonella* infection (EFSA, 2007a).

- 5.60 There is some indication in the literature that eggs laid by vaccinated chickens may be more resistant to *Salmonella* contamination and further multiplication, as maternal anti-*Salmonella* antibodies can be present in the egg (Hassan and Curtiss, 1996). In a recent study conducted in broilers, the samples collected from flocks that were progeny of vaccinated broiler breeders had a 62 % lower chance of being *Salmonella* positive than ones collected in equivalent flocks that were progeny of unvaccinated breeders (Berghaus *et al.*, 2011).
- 5.61 There is no doubt that the vaccination of laying hens against *Salmonella* Enteritidis across the EU has brought about a major improvement in public health.

***Salmonella* Enteritidis evolution**

- 5.62 Most *Salmonella* Enteritidis vaccines used in the EU are based on PT4. A key question to be addressed is whether the continued evolution of *Salmonella* Enteritidis challenges the efficacy of the PT4-based vaccines. Since 2002, the emergence of egg associated *Salmonella* Enteritidis PTs other than PT4 causing human infection has taken place in the UK, with the greatest increases occurring in *Salmonella* Enteritidis PT1 and PT14b cases (HPA 2004, 2005a). Surveillance of salmonellosis from 1998 to 2003 identified upsurges in non-PT4 *Salmonella* Enteritidis in other European countries (Fisher 2004). Most of the UK infections are thought to be associated with major changes in market supply including the importation of eggs from other egg producers in EU where there was a lack of vaccination of layer flocks against *Salmonella* or controlled assurance schemes in place (Fisher 2004, Nygård 2002, van Pelt *et al.* 2004) (see later in this chapter). It should be pointed out that EFSA data show marked improvements in the *Salmonella* Enteritidis status of laying flocks in EU states implicated in the UK outbreaks mentioned above. However, the risk from eggs sourced outside the UK continues to be measurably higher than those produced in the UK such as that provided by the British Lion mark certified farm assurance schemes. It currently seems that the introduction of a range of *Salmonella* Enteritidis PTs into the UK has not led to significant infection of laying flocks but there remains a research need to determine how well PT4-based vaccines protect hens against other *Salmonella* Enteritidis PTs.

Uncertainty

- 5.63 EFSA has recently published an extensive and authoritative document entitled “Guidance on Uncertainty in EFSA Scientific Assessment”. It examines, amongst many other factors less relevant to this chapter, human and technical errors or risks that may contribute to uncertainty about observed results. This

is clearly relevant to our task of trying to determine the true impact of the various interventions. In addition to the above EFSA document there is another entitled: “Guidance on Transparency and the Codex Working Principles for Risk Analysis”. Some of what follows has been taken from these two documents.

- 5.64 Uncertainty may be expressed qualitatively (descriptive expression or ordinal scales) or quantitatively (individual values, bounds, ranges, or distributions). It is not necessary or possible to separately quantify every individual source of uncertainty affecting an assessment. However, those trying to assess the robustness of data should always aim to express overall uncertainty in quantitative terms to the extent that is scientifically achievable, as is also stated in the EFSA documents referred to earlier. The principal reasons for this are the ambiguity of qualitative expressions, their tendency to imply value judgements outside the remit of assessors, and the fact that many decisions inherently imply quantitative comparisons (e.g. between exposure and hazard) and therefore require quantitative information on uncertainty.
- 5.65 There are many different stages and factors in the egg production chain that impact on the risk of *Salmonella* Enteritidis infection, from the individual hen that laid the egg, through housing systems, their hygiene and that of the farm environment, egg grading, packing and transport to retail outlets and finally how the consumer or caterer handle, store and use the eggs. Not all of these issues will be addressed in this chapter and our focus will be on the pre-retail or wholesale stages.

Under-reporting of human *Salmonella* cases

- 5.66 In the UK, cases of human *Salmonella* infection have shown a marked and sustained reduction since the late 1990s, in particular those caused by *Salmonella* Enteritidis. The numbers of *Salmonella* Enteritidis PT4 cases in the UK are now around the level they were before the international pandemic of egg-associated *Salmonella* began in the mid to late 1980s. Studies across the developed world have established that confirmed cases of *Salmonella* infection are always an underestimate of the true number, although the degree of under-estimation varies from country-to-country. However, there is no definitive evidence that under-reporting of human *Salmonella* Enteritidis cases in the UK has become more common, although changes to the NHS and surveillance bodies such as the Public Health Laboratory Service (PHLS), which is now part of Public Health England (PHE), and reductions in funding for local councils, may have had a negative impact on case recognition, the identification of *Salmonella* cases and the causative serovar.

- 5.67 Several Scientific Opinions from the EFSA BIOHAZ Panel related to setting of targets in poultry populations provide detailed information on underreporting of human salmonellosis (EFSA BIOHAZ Panel, 2010b, 2011, 2012). Details of the reporting system for human salmonellosis in the EU can also be found in the EU Summary Reports (EFSA and ECDC, 2010, 2011). The true incidence at population level may be considerably greater than reported, as discussed above, albeit that the level of underreporting varies between EU Member States (Member States; de Jong and Ekdahl, 2006). 'Multipliers' (i.e. the ratio between true and reported cases) can be found in scientific papers referring to single countries and, for example, range from 4.7 for the United Kingdom (Tam *et al.*, 2012), to 29.3 for the USA (Scallan *et al.*, 2011).
- 5.68 Underreporting values for human salmonellosis in the different EU Member States were estimated employing updated information on the risk from Swedish travellers in the EU in 2009, as described in detail by the EFSA BIOHAZ Panel (2011). The underreporting factor at EU level is estimated to be 57.5 (95 % confidence interval (CI) 9.0–172). For the EU-27, the estimated true incidence of salmonellosis in 2009 was estimated as 6.2 (95 % CI 1.0-19) million cases. The disease burden of salmonellosis and its sequelae is 0.23 million (95 % CI 0.05–0.6 million) disability-adjusted life-years (DALYs) per year and total annual costs were estimated at EUR 2 billion (95 % CI EUR 0.3–4 billion) (EFSA BIOHAZ Panel, 2011). It is estimated that in 2010 there were approximately 5.4 million (95 % CI 3.0-9.5 million) true cases of human salmonellosis in the EU-27, a 13 % decrease from 2009 (EFSA BIOHAZ Panel, 2012).
- 5.69 It should be noted that these data are only indicative, as the reported serovars often originate from different sampling schemes and there are differences among Member States in the way in which reports are made and the numbers of serovars reported. Travel, particularly outside the EU, is regarded as an important source of *Salmonella* Enteritidis (Tighe *et al.*, 2012)

Effect of temperature and duration of storage on the growth of *Salmonella* within eggs

- 5.70 One of the important requirements of the UK Lion scheme is that eggs are subject to temperature control from the farm until purchase by the consumer, at least in the major UK food retailers. Consumers are advised to store eggs under refrigeration until consumption. This is an important public health intervention. The storage conditions for fresh table eggs, has been the subject of much debate. Since *Salmonella* is thought not to be able to multiply in any foods at less than 7°C and is normally present at very low initial concentrations in fresh eggs, storage of eggs below this temperature, or even below 10°C,

would reduce the risk associated with eggs from an infected flock. However, even at 20°C, there is low level multiplication within eggs for 1-2 days after lay and after that high numbers of *Salmonella* Enteritidis in eggs are only achieved when the yolk membrane breaks down allowing the bacteria access to the nutrient-rich yolk, which takes around three weeks at 20°C (ACMSF Report 2005).

- 5.71 The rate of yolk membrane breakdown is temperature dependent and it is desirable to keep eggs as cool as practicably possible. It is especially important to avoid temperature fluctuations, as these accelerate membrane breakdown. There have been suggestions that the refrigeration of eggs in retail outlets, for example, would have a public health benefit by slowing the growth of *Salmonella* Enteritidis and the rate of yolk membrane breakdown. However, there is a danger of condensation on the shells once the eggs are removed from refrigerated storage by consumers. Wet egg shells can facilitate the movement of *Salmonella* Enteritidis into egg contents and survival on shells is prolonged in cool conditions, the risk associated with cooling eggs is likely to be small compared with the benefit, especially in hot countries with high levels of *Salmonella* in laying hen flocks (EFSA, 2014).
- 5.72 It is difficult to predict the rates of growth of *Salmonella* within eggs under different conditions since the prevalence of natural contamination is so low that experiments would be too expensive to be economic. Artificial inoculation of birds to produce a higher likelihood of internally contaminated eggs may rely on unnatural routes, such as high inoculation doses, immunosuppression or intravenous inoculation, so the equivalence to naturally contaminated eggs is open to question. Similarly, it is difficult to design experiments in which eggs can be artificially inoculated with low numbers of organisms without introducing biases, such as the presence of traces of culture media on the introduced cells or damage to cells in an attempt to remove this. Laboratory experiments can therefore only provide a general indication of the likely behaviour of *Salmonella* within eggs, and it is likely that human cases relate only to situations where, very rarely, an egg contains high numbers of organisms initially or there is temperature abuse of the eggs during storage or preparation of food.

Detection of *Salmonella* in eggs:

- 5.73 The main challenge concerning the detection of *Salmonella* Enteritidis in eggs is the typically very low rate of egg contamination, even among those originating from *Salmonella* Enteritidis-infected flocks. In addition, only very few *Salmonella* Enteritidis organisms are deposited within contaminated eggs in vivo. There is, however, a linear relationship between within-flock prevalence and the rate of contamination of egg contents, as well as a more

substantial contribution of prevalence to shell contamination (Arnold *et al.*, 2014a). In many countries, flocks of commercial laying hens are vaccinated against *Salmonella* Enteritidis which may further reduce the rate of contamination in eggs. These factors make the detection of *Salmonella* Enteritidis in raw eggs a challenging task.

- 5.74 There are other limitations in the ability to detect *Salmonella* in eggs, such as the number tested and sampling methods (Carrique-Mas and Davies, 2008). *Salmonella*-positive flocks of laying hens produce a small proportion of contaminated eggs (Humphrey *et al.*, 1989a; Davies and Breslin, 2003; Carrique-Mas and Davies, 2008; Arnold *et al.*, 2014a). With a low prevalence of individual egg contamination, large numbers of eggs have to be tested to obtain an accurate measure of egg contamination rates (Carrique-Mas and Davies, 2008). Furthermore, the production of contaminated eggs by *Salmonella* Enteritidis-infected hens has been found to be clustered and intermittent, in a study involving naturally infected hens (Humphrey *et al.*, 1989a).
- 5.75 The proportion of contaminated eggs, produced by an infected flock, depends on the within-flock prevalence. In a detailed approach, a within-flock model would describe the hen level dynamics which depend on the time since first infection and the interactions between hens and the transmission from hens to eggs. In a statistical analysis of empirical data on reported positive eggs (or pools of eggs) in samples from infected flocks, it is not known when the infection started in the flock or what the within-flock prevalence was at the time of sampling.
- 5.76 To detect *Salmonella* in batches of eggs where there is usually a very low expected prevalence of positives, especially in relation to egg contents it is necessary to sample 3000 eggs in order to reliably detect the expected 0.1 % prevalence of positive egg contents from an infected flock. Four thousand whole eggs (including shells), pooled in lots of 40, has been designated as an additional voluntary confirmatory sample for laying flocks for which a false-positive flock faecal sample is suspected (Regulation (EC) No 1237/2007). Samples taken from egg conveyors, candling and grading systems and the floor beneath such handling equipment provide a more sensitive indication of the prior passage of contaminated eggs. Gentle homogenisation of egg contents prior to culture in the laboratory increases the chance of detection, as it makes the yolk contents available to the bacteria and avoids sheer forces associated with vigorous maceration (Seo *et al.*, 2003).

- 5.77 In most situations, in order to avoid overwhelming laboratory resources, the contents of between six and forty eggs are pooled and cultured together. There might be some reduction in the test sensitivity due to a dilution effect derived from pooling eggs (Humphrey and Whitehead, 1992), although the precise magnitude of this effect is unknown. Because direct plating would not be able to detect fewer than 10^4 CFU/ml of *Salmonella*, additional steps or enhancements to the culture procedures are necessary. Pools of egg contents are either incubated or pre-enriched (or both) with or without supplementation of additives that promote the growth of *Salmonella* under the iron-limitation conditions that apply in egg albumen. In a recent study, (Pasquali *et al.*, 2013) tested the effect of pooling and of the initial contamination level on the detection of *Salmonella* on table eggs by the reference method ISO 6579. The authors found that the testing sensitivity on pooled samples was affected by the initial level of *Salmonella* contamination in the single positive egg, but not by the dilution of the positive egg with increasing number of negative ones. It is concluded that at least 16 pooled samples of 10 eggs each, characterised by a low prevalence and low contamination level, need to be tested in order to detect *Salmonella* with 95% certainty following the ISO 6579 method.
- 5.78 The albumen of the egg has a strong bacteriostatic effect due to the presence of ovotransferrin, which limits the amount of iron available to the bacteria and other anti-bacterial factors such as lysozyme (see Chapter 3). Iron supplementation during egg culture can therefore help overcome this effect, enhancing the growth of *Salmonella* and increasing the sensitivity of detection. This is especially necessary in situations in which abbreviated methods (i.e. incubation followed by direct plating) are used, rather than the three-step culture method using pre-enrichment. Iron salts such as ferrous sulphate have also been shown to promote the isolation of *Salmonella* Enteritidis from egg contents and may replace the pre-enrichment step of the culture, when added to the egg pool prior to incubation (Chen *et al.*, 2001). However, iron salts may also stimulate the growth of competing bacteria. Ferrioxamines, on the other hand, act by supplying *Salmonella* with useable iron, rather than saturating ovotransferrin, and therefore do not promote the growth of *E. coli* and the *Proteus–Providencia–Morganella* group, provided that they are not supplied at high concentration.
- 5.79 Because of the typically low prevalence of contaminated eggs, the low numbers of organisms in such eggs and the bacteriostatic effect of the albumen, multiplication of the relatively few organisms found in and on eggs is necessary to reach detectable levels in culture. This can be achieved using traditional three-step *Salmonella* culture methods. Longer periods of pre-enrichment (48 hours rather than 24 hours) have been shown to further increase the sensitivity

of detection in individual eggs or in pooled egg contents, as long as only a few competing organisms are present. In practice, in the EU, eggs are most likely to be tested as a foodstuff; therefore the full ISO 6579 method is used. Pre-enrichment at 41.5 °C may also be beneficial in some cases (Park *et al.*, 2012).

- 5.80 The detection of *Salmonella* Enteritidis, and other *Salmonella*, in egg contents, in particular, is complicated and time-consuming with many culture-related factors affecting the rate of isolation or the risk of laboratory contamination. It is important that methods validated by multi-centre trials are used by industry and other stakeholders.

Factors influencing detection of *Salmonella* infected flocks:

Detection methods

- 5.81 The efficiency of sampling programmes has a large impact on the detection of *Salmonella* and therefore estimation of prevalence of *Salmonella*-infected flocks (Fletcher, 2006). It has been recognised for some time that thorough environmental sampling is the most effective way to detect zoonotic serovars of *Salmonella* in a poultry flock (Aho, 1992; Johansson *et al.*, 1996; Musgrove and Jones, 2005). Boot or sock swabs are the easiest method for obtaining floor faecal samples from non-cage units, but pooled faecal droppings samples are used for caged flocks. Large hand-held gauze (or 'chiffonette') swabs can also be effective for sampling (Davies and Wray, 1996; Carrique-Mas and Davies, 2008; Zewde *et al.*, 2009) but more effort and dedication are required to achieve a representative sample. Detection of *Salmonella* in animal faeces and in environmental samples is used in the EU in primary production. Rapid detection methods such as PCR, gene probes and enzyme-linked immunosorbent assay (ELISA)-based tests have been described for use with poultry samples, but can be more subject to interfering substances in the sample (Jensen *et al.*, 2013) and inter-laboratory variation. So far no alternative methods have been approved for statutory use for monitoring food-producing animal populations in the EU, but several have been authorised in the USA (Adams *et al.*, 2013). Dust is a useful sample for identifying previous excretion of *Salmonella* by a poultry flock, especially in cage houses (Riemann *et al.*, 1998; Arnold *et al.*, 2014b). It is normally best to take both fresh faecal and dust samples (Davies and Wray, 1996) to help compensate for variable detection in either sample. In the EU Baseline survey of laying hens for *Salmonella* in 2004/2005, only one country, Ireland, had better detection of infected flocks via national monitoring than in the survey, and this was because routine sampling in that country was based on dust.

5.82 Immunological detection by serology can also be used to identify indirect evidence of likely exposure to *Salmonella* by detecting antibodies in serum or egg yolk (Davies *et al.*, 1997; Feld *et al.*, 2000). This increases the sensitivity of detection of those serotypes whose surface antigens are included in the ELISA-based test, normally *Salmonella* Enteritidis and *Salmonella* Typhimurium, compared with bacteriology alone, and a combination testing programme has been successfully used in Denmark for many years (Wegener *et al.*, 2003). Such testing cannot readily be used in vaccinated flocks but is a useful additional voluntary measure in non-vaccinated ones. Serological testing frequently detects false-positive reactions (Klinkenberg *et al.*, 2011) caused by exposure of birds to organisms with antigens that are shared with the target organisms, so such testing can only be used as an adjunct to bacteriological monitoring.

Sensitivity model and within flock prevalence:

5.83 After colonisation, individual laying hens shed *Salmonella* in their faeces intermittently, as determined by routine culture methods. Most hens stop shedding the bacteria after approximately three weeks (Shivaprasad *et al.*, 1990; Gast, 2005). However, under stress (water deprivation, viral or coccidial infection, stressful environments and moulting) the hens may resume shedding (Skov *et al.*, 2002). This can be explained by reactivation of shedding in latent carriers (Barrow, 1992) or by a higher susceptibility to re-infection from the environment (Skov *et al.*, 2002) as *Salmonella* Enteritidis, in particular, has a tendency to show long-term persistence in laying houses, possibly related to rodent levels and housing systems (Carrique-Mas *et al.*, 2008), as discussed below. In most poultry houses with vaccinated flocks, *Salmonella* Enteritidis and other serovars do not persist once rodents are eliminated (Davies and Carrique-Mas, 2010).

5.84 The within flock dynamics are expected to have a strong impact on the sensitivity of detection and consequently on model parameter estimates. This is because the within flock dynamics affect the within flock prevalence which is related to test sensitivity for a given flock (sensitivity is assumed to be <100%, but specificity is assumed 100%, even though sampling and laboratory contamination or mis-identification errors can occasionally result in false positive test results). Depending on the within flock situation (e.g. caged versus free range), the chances of detection can be different in different infected flocks (Arnold *et al.* 2014). Sensitivity of flock testing will depend, in part, on the samples taken. For, example, sampling dust is not better than boot swabs for detecting *Salmonella* in non-caged flocks but works better than faecal sampling for caged birds. For maximum sensitivity more than one sampling method

needs to be used in parallel and sampling of dust as well as faecal samples is recommended for epidemiological investigations.

- 5.85 Arnold *et al* (2013) found that the rate of egg shell contamination was higher in flocks with a high *Salmonella* prevalence, possibly linked to poor management. High prevalence makes a disproportionate contribution to the overall pattern of egg contamination. Rate of shell contamination was higher than for contents but *Salmonella* Enteritidis was the most frequent serovar isolated from contents. Schluz *et al* (2014) showed that the likelihood of finding *Salmonella* in positive flocks was not affected by flock age or season. Gole *et al* (2014) examined *Salmonella* shedding in a single aged caged flock at 18, 24 and 30 weeks. Positivity rates for faecal samples were 82, 39 and 13% for the three sampling times respectively, a clear pattern of decline as the birds aged. In this study, all egg belt and dust samples were positive throughout. Faeces collected from the lower tiers were significantly more likely to be *Salmonella*-positive than samples taken from higher cages. Serovars found in the study were Mbandaka, Worthington, Anatum and Infantis (Gole *et al*. 2014). In contrast to this work, several studies have shown an increased tendency for flocks to be identified as *Salmonella* positive as the birds become older (Garber *et al.*, 2003; van de Giessen *et al.*, 2006; Wales *et al.*, 2007; Bouzidi *et al.*, 2012; Roberts *et al.*, 2013) especially if birds have been moulted (Golden *et al.*, 2008), which is not common practice in the EU. In most cases, the initial infection results from residual contamination of laying houses that spread to pullets that are suffering from transport, handling and relocation/remixing stress at a time when hormonal changes associated with the onset of lay are also increasing susceptibility to infection (Line *et al.*, 1997). This leads to a typical early peak of infection within three weeks of housing (Humbert *et al.*, 1995; Gradel *et al.*, 2002) although laying flocks are rarely sampled at this time (16-19 weeks of age). There may also be an increase in shedding towards the end of lay, but in the absence of rodents this is less likely to occur and infection may spontaneously resolve (Carrique-Mas *et al.*, 2009).
- 5.86 Overall, it is clear that detection of *Salmonella* in laying flocks is far from straightforward, and some positive flocks will not be detected by any method, including the EU baseline survey and control programme methods that are used for confirmatory testing (Zenner *et al.*, 2013; Arnold *et al.*, 2014b). Since none of the sampling methods has a high level of sensitivity, any confirmatory sampling may possibly negate a previous positive result, even if there is no interference with the process by actions taken by the operator. Not all operator positives especially those positive for *Salmonella* Typhimurium are confirmed when official confirmatory sampling is carried out. A comparison of operator and official sample results provides an indication of some of the likely testing

deficiencies in monitoring programmes (Arnold *et al.*, 2010; Arnold *et al.*, 2014b) that may lead to under-detection of infected flocks. Some laying farms that were persistently infected with *Salmonella* Enteritidis in the years before control programmes were found to still be infected with the same strains when official sampling was carried out years later, suggesting infection may not have been detected by tests carried out in the intervening years. The significance of under-detection in terms of public health is unclear, as it is the most highly infected flocks that are likely to be detected, and eggs from flocks with low levels of infection are less likely to be contaminated (Van Hoorebeke *et al.*, 2009). However, there is still some way to go before *Salmonella* Enteritidis infections in the European population, particularly outside the UK, reach the low levels of the pre-epidemic period in the 1970s.

The impact of housing and production systems

5.87 One major change since our previous report was written (2005) is that new EU welfare legislation, driven largely by consumer pressure, has led to a ban on the use of conventional 'battery' cages for laying hens since January 2012. This means that cage houses have been decommissioned or refurbished to provide an alternative housing system. Such alternative systems, which involve smaller flocks, are less conducive to *Salmonella* infection. Some cage houses may be converted to barn production, typically as two-storey barns or aviaries, but the most likely option is conversion to enriched colony cages, in which groups of 30–80 birds are housed in a larger cage that provides more space, perches and a 'nest-box' area. Conversion of houses required mass removal of old-style cages (Van Hoorebeke *et al.*, 2011), which offered an excellent opportunity to eliminate farm pests that can carry *Salmonella*, such as rodents, flies and litter beetles, as well as red mites, which can reduce the resistance of birds to *Salmonella* infection in the case of heavy infestations (Wales *et al.*, 2010). During the extended down-time involved in refurbishment, houses can be deep cleaned and intensively disinfected to remove residual environmental contamination. This was a great opportunity to eliminate resident *Salmonella* from cage houses, and reduce infection risk (van Hoorebeke *et al.*, 2012). Colony cage nest and perch areas can be more difficult to clean than conventional cages but the belt-cleaning system means that there is less harbourage for rodents and flies than in the deep pit houses that they replace. Despite the fact that numerous risk factors associated with colonisation have been identified and quantified, and several control measures have been implemented (Galiş *et al.*, 2013), introduction of *Salmonella* into flocks may still occur, possibly at a lower frequency than before (van de Giessen *et al.*, 2006). Some of the cases of apparent new infections may also be examples of chance detection of infections that were previously below the limit of detection.

- 5.88 Currently there is no consensus regarding the impact of caged, barn and free range egg production on *Salmonella* contamination of eggs. Publications assessing the impact of various methods of egg production on *Salmonella* contamination are conflicting, which makes it difficult to implement informed legislation to ensure food safety. Studies comparing *Salmonella* contamination in the different egg production processes have yielded conflicting and inconsistent evidence, probably because of the complexity of confounding factors and variables. These factors include flock size, flock age and stress caused by re-housing, weather, transport and stage of lay.
- 5.89 An EFSA study tested faecal and dust samples from 5000 egg production sites across 25 European countries and concluded that cage flock holdings were more likely to be contaminated with *Salmonella*. However, a more recent review by Holt *et al.* (2011) concluded there was no general consensus as to which egg production housing system resulted in less *Salmonella* contamination. This review was criticised by Greger (2011) who stated that Holt *et al.* (2011) had misrepresented EFSA data by only citing individual studies from only a few countries in the study. Such work demonstrates the complexity of this issue and indicates that there is not a single answer, although the standards of rodent control (Buckle and Smith, 2015) and origin of birds in different countries has a major impact on risk in different housing systems.
- 5.90 As discussed earlier in this report, the infection dynamics of *Salmonella* Enteritidis may depend on a number of factors (Howard *et al.*, 2005) and housing systems and flock management are important in this process. Risk factors included flock size (Mollenhorst *et al.*, 2005; EFSA, 2007b; Namata *et al.*, 2008; Huneau-Salaun *et al.*, 2009) and the size of the farm, which is also linked with hygiene practices, as large farms are more likely to be dry cleaned only, rather than washed and disinfected, between flocks (Aimey *et al.*, 2013). On-floor housing systems (Garber *et al.*, 2003; Mollenhorst *et al.*, 2005) and cage systems (EFSA, 2007b; Namata *et al.*, 2008; Gast *et al.*, 2013) were found to increase the risk of colonisation of flocks by *Salmonella* or of egg contamination by these or other bacteria (De Reu *et al.*, 2009; Jones *et al.*, 2012) in some studies, but to have no influence in others. These contrasting findings are likely to relate to national variations in housing systems, management and sources of birds, but, in general, cage production has been found to be associated with an increased risk of flock infections by *Salmonella*, and non-cage systems result in dirtier eggs, which are more likely to be contaminated by pathogens if the flock is infected (Holt *et al.*, 2010). The age of the poultry house is also a significant risk factor, as *Salmonella* Enteritidis may persist in laying farms for decades. Multi-stage management in on-floor

flocks was also identified as a risk factor ((Mollenhorst *et al.*, 2005; Huneau-Salaun *et al.*, 2009) and the finding of generally lower risk probably relates to the greater use of all-in/all-out systems and smaller numbers of flocks and birds in non-cage systems.

- 5.91 It is unfortunate that there are few studies investigating the effect of different housing systems on egg contamination. Gast *et al.* 2013 compared *Salmonella* contamination of hens in conventional cages and colony cages enriched with perching, nesting and scratching areas. Hens were orally dosed with 1.0×10^7 CFU of *Salmonella* Enteritidis for five to six days prior to euthanasia and testing of internal organs. *S. Enteritidis* was detected at significantly higher frequencies in the livers, spleens, ovaries and oviducts of the hens housed in the conventional cages compared to those in enriched ones. It was suggested to be due to housing parameters such as stocking density or behavioural attributes which might affect the susceptibility of hens to disseminated infection. However, another study by Gast *et al.* (2014) demonstrated experimentally that there were no significant differences in the rates of transmission of *Salmonella* Enteritidis from infected hens to healthy ones housed in conventional or enriched cages. In a more recent study, Gast *et al.* (2015) using artificial infection with *Salmonella* Enteritidis PT4 and PT13a, reported that birds in conventional cages were significantly more likely to be faecal positive for either PT.
- 5.92 The effect of housing on the transmission of *Salmonella* Enteritidis infection was also explored by De Vyllder *et al.* 2011. Four housing systems were tested using experimentally infected hens. This included a conventional battery cage, a furnished cage (most similar to an enriched cage), an aviary, and a floor system. The spread of infection between hens was slightly more in the aviary and floor housing systems compared to the two caged housing systems. This was partly reflected in egg contamination as significantly more contaminated eggs were found in the aviary housing systems compared to two cage and floor housing systems. It was suggested that the increased spread of infection could be linked to inherent differences between the housing systems, including hygienic status, air quality and increased physical contact between birds. However, in a review, Van Hoorbeke *et al.* (2010) examined the influence of chicken housing systems on *Salmonella* infections. Based on epidemiological data the authors concluded that it is highly unlikely that the move from traditional caged systems to enriched cage and non-cage methods of production will increase the risk of *Salmonella* infection and shedding by the animals. However, the authors recognise that there are many confounding factors such as bird age, age of the infrastructure and rodents etc.

Environmental contamination:

- 5.93 Exposure to a *Salmonella*-contaminated environment is clearly an important factor in determining whether laying hens will become infected with *Salmonella* Enteritidis or other *Salmonella* serovars. Some studies suggest that environmental sources present in free range housing have a lower incidence of *Salmonella* contamination compared to caged housing. A Belgian study found that 30% (45/148) of dust samples and 30% (45/148) of faecal samples collected from caged housing were positive for *Salmonella*; whereas, only one out of 148 of dust samples and two out of 148 faecal samples collected from barn and free range housing were positive for *Salmonella* (Van Hoorebeke, 2009). These results were supported by a UK study by Wales *et al.* (2007) who found the incidence of *Salmonella* in environmental samples to be higher in caged housing (19%) than in free range systems (10%). A study by Recio *et al.* 2007, which investigated the presence of *Salmonella* Enteritidis in faeces and dust samples from 5310 egg production holdings across the EU found that free range housing systems had significantly lower *Salmonella* contamination compared to caged housing systems. However, conflicting evidence was presented by Parisi *et al.* 2015 who used 84 certified *Salmonella*-free Bovan Brown hens to experimentally demonstrate that free range eggs had a higher incidence of *Salmonella* contamination compared to conventional battery cages. In this study 5/212 (2%) eggs sampled from three free range housings and 0/212 from three conventional battery cages tested positive for *Salmonella*. It was suggested that the higher *Salmonella* incidence in the free range housing was due to prolonged contact between the hen/nest box and the egg after it has been laid and less clean conditions, compared to cage systems in which the egg is removed more quickly from the physical proximity to the hen.
- 5.94 The *Salmonella* serovar, or the strain of a particular serovar, will have had an impact on the data produced from the above experiments. As we say earlier in this report, the behaviours of one strain of *Salmonella* Enteritidis PT4, for example, does not necessarily indicate the behaviour of others. Hen type and the age and health of the breeder flocks that produced them are also potentially important confounding factors.

The role of non-UK eggs in *Salmonella* Enteritidis infections/outbreaks in the UK

- 5.95 The public health risk can normally be assessed for UK-produced eggs but the true status of eggs sourced from some other countries is open to question, despite national monitoring data (Little *et al* 2006). Surveys and investigations of eggs for *Salmonella* contamination have played an important role in

understanding the extent and pattern of contamination. Studies of eggs appear to indicate that those originating from some countries outside the UK have a higher rate of *Salmonella* contamination than UK-produced eggs. In 1996/97, a survey of non-UK eggs intended for retail sale found that 2% of samples contained *Salmonella* 1.3% contained *Salmonella* Enteritidis and 0.1% contained *Salmonella* Enteritidis PT4 (ACMSF 2001). The Health Protection Agency (HPA) outbreak-associated examination of eggs during 2002 to 2004 showed a higher rate of *Salmonella* contamination in or on eggs from outside the UK and used in catering premises. Most *Salmonella* isolates were *Salmonella* Enteritidis non-PT4 (5.5% in Spanish eggs; 6.3% in eggs of country of origin not known) (HPA 2004, Little *et al.* 2006). In contrast, rates of *Salmonella* contamination in UK-produced eggs were shown to have decreased significantly, clearly demonstrating an improved situation (1995/6; 1.0%, 2003; 0.3%) (ACMSF 2001, Elson *et al.* 2005, FSA 2004a).

- 5.96 A FSA survey of *Salmonella* contamination of non-UK eggs on retail sale was carried out over a period of 16 months, between March 2005 and July 2006. The main objectives of the survey were to estimate the prevalence of *Salmonella* in non-UK raw shell eggs at retail sale and to identify the *Salmonella* serovars and PTs. The study also investigated associations between types of *Salmonella* and the country of origin. Two-thirds (66.3%) of eggs sampled were from Spain, 20% from France, 7.4% from The Netherlands, 2.6% from Germany, with the remainder of the eggs originating from Portugal, Republic of Ireland, Belgium and Poland. The overall finding was that 157 samples were contaminated with *Salmonella* on the shell of the egg resulting in a weighted prevalence estimate of 3.3%, which is equivalent to 1 in every 30 'boxes' of 6 eggs. Of these, *Salmonella* Enteritidis was detected in 136 samples with a prevalence estimate of 2.6%, which is equivalent to 1 in every 40 'boxes' of 6 eggs. Of the 157 *Salmonella* shell positive samples, 10 were also "contents positive" (6 samples also contained two separate *Salmonella* isolates) making a total of 173 distinct *Salmonella* isolates recovered. From these eight different serovars were found, most of which were *Salmonella* Enteritidis (84.9%; 147/173). There were nine *Salmonella* Enteritidis PTs, with PT1 predominating (81.6%; 120/147). *Salmonella* Enteritidis PT4 was not detected. Other serotypes found included S. Mbandaka (14), *Salmonella* Unnamed (6), *Salmonella* Rissen (2), *Salmonella* Braenderup (1), *Salmonella* Infantis (1), *Salmonella* Panama (1) and *Salmonella* Weltevreden (1). The majority of the *Salmonella* isolates were resistant to one or more antimicrobial drugs (83.2%) of which most were resistant to nalidixic acid with reduced susceptibility to ciprofloxacin (78.6%).

- 5.97 Most of the positive egg samples (6 eggs) were from Spain (66%) or France (20%). A small proportion (5.9%) of samples was produced from laying hens vaccinated against *Salmonella* or under a controlled assurance scheme, none of which were contaminated with *Salmonella*. The authors state that “Vaccination of layer flocks, or those certified as free from *Salmonella* Enteritidis and *Salmonella* Typhimurium under controlled assurance schemes, combined with improved biosecurity, does appear to have had a significant impact on the prevalence of *Salmonella* Enteritidis PT4 contamination of eggs and on human *Salmonella* infection (ACMSF 2001, Grein *et al.* 1997). Continued surveillance of human and veterinary salmonellosis is essential to detect emerging and future problems”.
- 5.98 Additional information on the packaging for 102 egg samples indicated that they had either been produced from laying hens vaccinated against *Salmonella* (n=12) or from a KAT (Association for Controlled Alternative Animal Husbandry) controlled assurance scheme (n=90). The eggs from laying hens vaccinated against *Salmonella* were cage eggs produced in Spain, while the KAT controlled eggs were free range eggs produced mainly in The Netherlands (89 samples from The Netherlands, 1 sample from Belgium). *Salmonella* were not detected from eggs produced under either of these egg assurance schemes.
- 5.99 These authors also conducted other studies. Little *et al* (2008) sampled eggs from catering premises in the UK in 2005-6. *Salmonella* was detected in 0.4% of UK-produced eggs and in 2.6% of eggs from Germany. In most cases contamination was only on the shell. Little *et al* (2007) tested eggs in catering establishments between 2002 and 2004. *Salmonella* was recovered from 3.4% of 16971 eggs tested. 5.5% of Spanish eggs were positive and 6.3% of positive eggs were of unknown origin. 1.1% of non-Lion UK eggs were positive and no *Salmonella* were recovered from eggs produced under the Lion scheme, although relatively few were tested.
- 5.100 These reports highlight the public health dangers of using eggs not produced under robust industry control schemes and, once again, indicate the benefits to consumers and caterers of the vaccination of laying hens against *Salmonella* Enteritidis and *Salmonella* Typhimurium. While control in many EU member states has improved since the above work was performed there are still UK outbreaks being reported that involve non-UK produced eggs.

Conclusions

- 5.101 Since *Salmonella* is thought not to be able to multiply in any foods at less than 7°C and is normally present at very low initial concentrations in fresh eggs, storage of eggs below this temperature, or even below 10°C, would reduce the risk associated with eggs from an infected flock. However, even at 20°C, there is low level multiplication within eggs for 1-2 days after lay and after that high numbers of *Salmonella* Enteritidis in eggs are only achieved when the yolk membrane breaks down allowing the bacteria access to the nutrient-rich yolk, which takes around three weeks at 20°C (ACMSF Report 2005).
- 5.102 The rate of yolk membrane breakdown is temperature dependent and it is desirable to keep eggs as cool as practicably possible.
- 5.103 It is likely that human cases of salmonellosis relate only to situations where, very rarely, an egg contains high numbers of organisms initially or there is temperature abuse of the eggs during storage or preparation of food.
- 5.104 The detection of *Salmonella* Enteritidis, and other *Salmonella*, in egg contents, in particular, is complicated and time-consuming with many culture-related factors affecting the rate of isolation or the risk of laboratory contamination. It is important that methods validated by multi-centre trials are used by industry and other stakeholders.
- 5.105 There may also be an increase in shedding towards the end of lay, but in the absence of rodents this is less likely to occur and infection may spontaneously resolve.
- 5.106 There is still some way to go before *Salmonella* Enteritidis infections in the European population, particularly outside the UK, reach the low levels of the pre-epidemic period in the 1970s.
- 5.107 Studies of eggs appear to indicate that those originating from some countries outside the UK have a higher rate of *Salmonella* contamination than UK-produced eggs.
- 5.108 Continued surveillance of human and veterinary salmonellosis is essential to detect emerging and future problems.
- 5.109 While control in many EU member states has improved since the above work was performed there are still UK outbreaks being reported that involve non-UK produced eggs.

5.110 This section has identified many of the potential confounding factors that can make it difficult to gain a robust picture of the incidence of bird infection with *Salmonella*, egg contamination rates and/or the true incidence of human infection. It is essential that comparisons of results from different experiments/surveys take into account a range of relevant technical factors including sampling and microbiological testing methods, along with wider aspects such as the particular *Salmonella* strain used to infect birds and the conditions under which the animals were housed. There is no doubt that the marked reduction in the incidence of *Samonella* Enteritidis in people, laying flocks and eggs in the UK is real and the reduction of *Salmonella* Enteritidis in Europe is a contributory factor to this. We can be less certain about the accuracy of the reported prevalence for laying flocks across Europe, and the current incidence of contamination in eggs sourced from EU countries.

Chapter 6: Revisiting the risk assessment model. Have all the data gaps identified in 2001 been filled?

The FSA risk assessment model

- 6.1 In 2001 the FSA developed a prototype quantitative risk assessment for *Salmonella* Enteritidis in eggs. The assessment was based on collected evidence from the UK (literature and opinions) and was implemented as a spreadsheet model (with accompanying flow diagrams to illustrate the model domain and the influence of information). The FSA prototype risk assessment model is reported in the ACMSF second report on *Salmonella* in eggs.
- 6.2 The FSA risk assessment represented *Salmonella* Enteritidis contamination of flocks, eggs and foods for consumption but did not include dose-response modelling (i.e. it did not extend to cases of human illness). The assessment was segmented according to three production types (barn, battery and free range) and included a separation of non-UK eggs. The assessment represents a fixed time (i.e. it does not include dynamics such as shifts in dominant production methods or surveillance results).
- 6.3 Alongside the development of a UK risk assessment model the FSA concluded, in 2001, that an existing US FSIS assessment for *Salmonella* Enteritidis in eggs (*Salmonella* Enteritidis risk assessment. Shell eggs and egg products. FSIS/FDA final report 1998 - <http://www.fsis.usda.gov/OPHS/risk/contents.htm>) was not sufficient for the UK because it did not map all methods of contamination, it did not account for all production methods and it did not consider vaccination practices used in the UK.
- 6.4 In 2009 an updated FSA exposure assessment model was presented to the ACMSF (ACM/937). This model also has a spreadsheet (Monte Carlo simulation) implementation. The model is segmented into four production types (barn, battery, free range and organic) and into two quality codes (Lion and non-Lion). The model follows the *Salmonella* contamination from egg production to individual servings of (prepared) food.
- 6.5 The FSA *Salmonella* in eggs exposure assessment model can be considered as four modules representing production, retail, preparation and cooking. The construction uses probabilities to represent uncertain quantities and combines them to express belief about output measures. The probability of eggs being contaminated at the point of sale and the probability of an egg based meal being contaminated at the point of consumption are key outputs.

- 6.6 The module for UK egg production combines 'between flock' (EU surveillance 2004-2005 <http://www.efsa.europa.eu/en/efsajournal/pub/310ar>) and 'within flock' (VLA epidemiological research 2000-2003) prevalence estimates, for *Salmonella* contamination, to give the probability that an individual laying hen is contaminated. In turn this probability is combined with estimates for *Salmonella* transmission rates, from the bird to the interior of the egg or from the bird to the exterior of the egg (VLA epidemiological research 2000-2003), to give the probability of *Salmonella* in an egg at the point of lay. The prevalence estimates discriminate over production method and the transmission rates discriminate over the quality code (vaccination status).
- 6.7 The module for UK egg production also includes a complex model for cross contamination, i.e. the probability for *Salmonella* to transfer from the environment to the exterior of the egg (VLA epidemiological research 2000-2003 and additional VLA expert opinion about lay house contamination) and the probability for *Salmonella* to transfer from the exterior to the interior of the egg (expert opinion from the assessors). These processes are effective in both the laying house and the packing house. Cross contamination is assumed to be independent from the production method or the quality code.
- 6.8 The FSA exposure assessment egg production module uses survey data (FSA 2005-2006 <http://webarchive.nationalarchives.gov.uk/20120206100416/http://food.gov.uk/multimedia/pdfs/nonukeggsreport.pdf>) to establish the rate for *Salmonella*, both interior and exterior contamination, associated with non-UK eggs. The survey identifies three sources for non-UK eggs: Spain, France and Other (all the eggs testing positive for *Salmonella* originated from Spain).
- 6.9 The retail module of the FSA exposure assessment assumes that additional eggs may become contaminated during the transition from the packaging environment to the point of sale. The model for additional contamination of egg exteriors includes both direct contact events, involving a contaminated egg, and indirect contamination caused by reuse of packaging materials (egg trays). All the probabilities, including transfer rates, were estimated with uncertainty (opinion established in consultation with the FSA 2006) as part of the assessment. The model for transfer of *Salmonella* from the exterior to the interior of the egg, established in the production module, is used again within the retail module to update the probability of contamination within the interior of an egg. Changes in contamination during the transition from packaging to point of sale are independent of the production method or the quality code.

- 6.10 The retail module of the FSA exposure assessment for *Salmonella* in eggs includes UK market and retail information (proportions without uncertainty from the BEIS survey 2006 <https://www.egginfo.co.uk/egg-facts-and-figures/industry-information/data> and DEFRA egg statistical notice https://data.gov.uk/dataset/egg_statistics_notice) so that it is possible to estimate the probability of *Salmonella* contamination, and the exterior and interior components, for commercial operations such as catering or manufacture of egg products and for each category of retail outlet (the model development includes an element of validation based on the FSA retail survey of shell eggs 2005-2007 <http://webarchive.nationalarchives.gov.uk/20120206100416/http://food.gov.uk/multimedia/pdfs/nonukeggsreport.pdf>).
- 6.11 The retail module of the FSA exposure assessment for *Salmonella* in eggs includes survey results (FSA retail survey of shell eggs 2005-2007 and DEFRA report 2006) so that it is possible to estimate the probability of *Salmonella* contamination, and the exterior and interior components, from eggs sourced outside the UK with a corresponding segmentation by retail and catering uses and by overseas source.
- 6.12 UK import statistics (DEFRA egg statistics 2000-2007) provide weights so that it is possible to estimate the probability of *Salmonella* contamination, and the exterior and interior components, for all eggs at the point of sale in the UK (estimated as 8.6 billion eggs in 2006).
- 6.13 The food preparation module of the FSA exposure assessment for *Salmonella* in eggs uses expert opinions (FSA microbiological safety division 2009), and some uninformative beliefs to establish the rate at which egg contents are contaminated from the exterior of the egg, and from other sources such as utensils, when the shell is broken and during preparation. Additionally survey results (Department of Health survey of egg shells 2005-2007) are used to estimate the extent to which contaminated interiors are mixed (pooled) when preparing eggs for consumption. The rates at which eggs are prepared by different cooking techniques are established from an expert elicitation exercise (Department of Health questionnaire for the ACMSF 2000). The rates are segmented according to the domestic or catering environment of the egg preparation. The rates can be combined to estimate the probability that a serving, prepared in a particular fashion, is contaminated with *Salmonella* prior to cooking.
- 6.14 The cooking module of the FSA exposure assessment for *Salmonella* in eggs uses expert opinions (Department of Health questionnaire for the ACMSF

2000) to estimate the rate at which egg are undercooked. This information is combined with information about pooling of eggs during preparation to establish the rate at which servings of egg are undercooked (contaminated). The rates are segmented according to the domestic or catering environment of the egg preparation and according to the method of cooking.

- 6.15 In 2009 the FSA exposure assessment for *Salmonella* in eggs indicated that, at point of sale, median values for *Salmonella* contamination of all, UK and imported eggs were 0.115%, 0.062% and 0.671%. At the point of consumption the median values for the *Salmonella* contamination rates of all eggs, domestically prepared eggs and eggs from catering services were 0.030%, 0.018% and 0.035%.
- 6.16 Follow on analysis (sensitivity analysis) by the FSA identified, in 2009, five key inputs, for the exposure assessment model, that have a significant influence on the model outputs; (i) the within flock prevalence (ii) the rate at which laying houses are contaminated with *Salmonella* (iii) the rate of contamination, for the exterior of the egg, in the laying house and in the packing house (iv) the rate of contamination of eggs during food preparation, that arises from sources other than the exterior of the egg, (v) the rate at which egg based foods are undercooked. Some scenario analysis by the FSA, conducted with the FSA exposure assessment model and communicated to EFSA in 2009, indicated that reduction in the contamination rate of non-UK eggs to the UK would have the largest impact on contamination at the point of sale i.e. analysis indicated that the removal of contamination from those eggs would lead to a 50% reduction of the baseline rate for contaminated eggs at point of sale.

Additional considerations

- 6.17 Additional considerations, relevant to exposure assessment for *Salmonella* in eggs, arise from updated surveillance and from new research results. Regular EU and UK monitoring of *Salmonella* in laying hens are well established and could support a dynamic extension to exposure assessment (The EU Summary report on zoonoses, zoonotic agents and food borne outbreaks 2013 indicates a between flock prevalence ~0.92% for *Salmonella* in UK laying hen flocks c.f. 23.8% for battery flocks considered by the FSA exposure model). Equally, changes in volumes of eggs sourced from other EU countries (and locations) and changes in production methods for eggs also contribute to changes in exposures.
- 6.18 Additional considerations, relevant to exposure assessment for *Salmonella* in eggs, arising from research include improved, quantitative, understanding of

egg contamination rates (e.g. Arnold *et al.* 2014), additional modelling of survival for *Salmonella* on table eggs (Pasquali *et al.* 2016), improved understanding of internalization and growth of *Salmonella* in eggs (e.g. De Winter *et al.* Int. Food Risk Analysis 1, 40-81, 2011, Singh *et al.* Journal of Food Science, 76, M225-M232, 2011) and, particularly, improved understanding of the time-temperature related breakdown of the internal membrane and the relationship with the probability of microbial population growth. These steps indicate that details of egg storage and handling conditions may be relevant for improved exposure assessments for *Salmonella* in eggs (and shelf life related decisions).

Other risk assessment models

- 6.19 In a recent opinion the EFSA BIOHAZ panel has considered risks, and risk assessments, of table eggs due to deterioration and development of pathogens (EFSA Journal 12(7), 3782, 2014). Within the opinion the BIOHAZ panel reviewed several quantitative risk assessments, for *Salmonella* in eggs, which were published in the period 2002-2011; these include assessments developed in Finland, Ireland, the USA, Canada and Australia. The EFSA BIOHAZ panel selected an Australian assessment as the starting point for consideration of *Salmonella* risks, from eggs, in the EU.
- 6.20 The modified Australian eggs storage model developed by the EFSA BIOHAZ panel has many features in common with the FSA exposure assessment but, additionally, includes a (Poisson) model for the number of *Salmonella* cells in eggs at lay, a (cardinal value) model for the growth of *Salmonella* in eggs (based on a model developed by Singh *et al.* "Dynamic predictive model for growth of *Salmonella* in liquid whole egg" J. Food Sci. 76, M255-M232), a kinetic (time-temperature) model for the breakdown of the vitelline membrane in eggs model (identified with Whiting *et al.* "A quantitative process model for *Salmonella* Enteritidis in shell eggs" J. Food Science, 65, 864-869, 2000 but originating with Humphrey "Contamination of egg shell and contents with *Salmonella* Enteritidis: A review" Int. J. Food Micro 21, 31-40, 1994) and a beta-Poisson dose response model (based on outbreak studies) for human illness caused by *Salmonella*. The modified model is implemented as a Monte Carlo simulation and has been used to explore the effects of distinct time temperature storage scenarios for eggs. The EFSA modelling includes changes in the use of eggs, following different communication strategies, and considers the role of microbial spoilage and egg quality indicators.
- 6.21 The modified Australian eggs storage model developed by the EFSA BIOHAZ panel does not include details (mechanics) of the production process (i.e. cross

contamination in the laying house, since this does not affect changes during subsequent storage) and is only concerned with the internal contamination of eggs, as external contamination reduces during storage and no quantitative data is available for shell contamination or for the theoretical possibility of trans-shell migration. Most crucially, the model developed by the EFSA BIOHAZ panel describes relative risks (i.e. comparisons of storage scenarios in terms of additional illnesses) rather than absolute numbers of illnesses (relative risks are less sensitive to uncertainties in the inputs to risk assessment).

- 6.22 Most recently another mathematical model, that concentrates on the impact of the time and temperature of storage on *Salmonella* growth in table eggs, was published by BfR from Germany (Gross *et al.* "Salmonella in table eggs from farm to retail – when is cooling required" Food Control, 47, 254-263, 2015). This assessment centres on a model for the yolk membrane time and on bacterial growth following membrane deterioration. The German construction includes a quantification of the membrane breakdown that is similar to the one used in the modified Australian model and a Ratkowsky (secondary) model for the maximum specific growth rate (attributed to Whiting *et al.* 2000). For storage in sequential isothermal environments the model constructs the cumulative fraction of the expired yolk membrane time as the determinant of population growth for *Salmonella*. This assessment uses scenario analysis to support cooled storage of eggs that have extended lifetimes.

Recommendation

- 6.23 It would be practical, and advantageous, to bring together the FSA exposure assessment model with updated data from UK surveillance and with the modified Australian model, proposed by the EFSA BIOHAZ panel, to form an improved assessment for hazards that are associated with *Salmonella* in eggs in the UK.

Chapter 7: Role of different *Salmonella* Serovars in egg contamination

Background

- 7.1 There are over 2,600 different *Salmonella* serovars, and many strains of each serovar. Food animals such as laying hens may be exposed to many different types via feed and/or environmental contamination during production and may become colonised. While most of these salmonellae are confined to the gut of the animals, movement to the reproductive tract is possible as the vagina is very close to the cloaca. These *Salmonella* types do not seem capable of long-term infection of reproductive tissues and if they contaminate eggs at all, the bacteria are confined to the egg shell and pose only a low public health risk, if any.
- 7.2 There are a small number of *Salmonella* serovars which are considered to have an enhanced ability for long-term colonisation/infection of the reproductive tract of laying hens. From a human health perspective *Salmonella* Enteritidis is the most important. Most *Salmonella* infecting the avian reproductive tract are host adapted, such as *Salmonella* Gallinarum biovars Gallinarum and Pullorum and the turkey-associated O18 *arizonae* serovar (Anon, 2012 a,b). None of these have been identified in commercial scale egg production in GB for several years and their zoonotic potential is considered to be very low, with infections only relating to severely immunocompromised people, and such infections are hardly ever reported.
- 7.3 A second category of *Salmonella* involves strains that are more likely to be capable of systemic infection, rather than remaining localised in the intestine and gut-associated lymphoid tissue. For both these categories there is an association with specific serovars, but within these serovars only certain genovars or lineages possess this potential, whilst others may be non-invasive or even host-adapted to non-human animals. Furthermore, even within phage types such as *Salmonella* Enteritidis PT4, there is considerable variation in the infectivity for hens, persistence within hens, transmission into forming eggs and survival in egg albumen (Shah *et al.*, 2012). It is therefore an over-simplification to consider the behaviour of strains within a serovar to be equivalent. However, for practical disease control purposes it is not possible to readily identify the infection-potential of strains, and vaccines for chickens are designed to be active against specific serovars, so it makes practical sense to consider a serovar as a distinct entity.
- 7.4 Common sense dictates that *Salmonella* types able to infect and persist in the avian reproductive tract and contaminate forming eggs, *in vivo*, must have a

particular genetic make up to allow them to do this. Surveys of eggs carried out in Europe have revealed *Salmonella* Enteritidis as the predominant serovar associated with eggs, as earlier chapters of this report have indicated. We discuss *Salmonella* Enteritidis and the genes it has that allow it to pose the particular egg-associated egg threat that it still does in many parts of the world, and make reference, as appropriate to Chapter 3. Our focus will be on other 'egg-associated *Salmonella*' identified in surveys and outbreak investigations and will compare, where possible, their *in vivo* behaviours in eggs and chickens with that of *Salmonella* Enteritidis. The threat in the UK from *Salmonella* Enteritidis in/on eggs is now very low and has been for over 10 years but other 'egg-associated *Salmonella*' may pose a future health threat, particularly if no vaccine is available and the strains have acquired plasmid mediated virulence genes (Johnson and Lang, 2012, Threlfall *et al.*, 2014).

- 7.5 Earlier chapters of this report and our document on *Salmonella* in eggs (ACMSF 2005) established that *Salmonella* Enteritidis was overwhelmingly the most important serovar in human egg-associated salmonellosis worldwide, and that *in vivo* egg contents contamination was the most important factor in transmitting *Salmonella* Enteritidis to people. The key question to be addressed in this chapter is whether there are other *Salmonella* serovars that could have similar properties and human health impact to *Salmonella* Enteritidis. As we discuss briefly above, there are probably 4-5 *Salmonella* serovars that contain strains that are capable of persistently infecting the hen's reproductive tract and being transmitted into egg contents. *Salmonella* is common in animal production systems, and surveys of broiler chickens and laying hens over the last 40 years revealed that the birds were infected or colonised by a variety of serovars. Similarly, the production environment was often *Salmonella*-positive. For both birds and their environment there has been a marked fall in *Salmonella* levels in the UK, and much of the rest of the EU, particularly in the last 25 years. Testing of eggs in the EU between 2004 and 2012 found over 20 *Salmonella* serovars on egg shells with *Salmonella* Enteritidis being the most common and *Salmonella* Typhimurium being the second most frequently found serovar.
- 7.6 The above data suggest that *Salmonella* Enteritidis and *Salmonella* Typhimurium pose the greatest threat to public health, although it is our view, supported by multi-national epidemiological data, that the threat from the former greatly outweighs that from the latter in relation to eggs. However, given the importance of *Salmonella* Typhimurium it is also discussed below. A small number of outbreaks associated with "other" *Salmonella* serovars are reported by EFSA each year to be associated with eggs, but these are often related to egg products that can contain additional food ingredients or be subject to cross-contamination from other sources during preparation. The extent to which shell

contamination can contribute to egg-borne infection is unknown, but in view of the low prevalence and low numbers of organisms found on eggshells, human infection could only result from cracking and pooling of eggs and subsequent storage temperature abuse. The possibility of egg shells contaminating staff and kitchen equipment is also unquantified, but would also be expected to represent a low risk.

- 7.7 ***Salmonella* Typhimurium:** In a report by Whiley and Ross (2015) it is stated that “*in the United States between 1985 and 2002 contamination of eggs was identified as the source of 53% of all cases of Salmonella reported to the Centre for Disease Control and Prevention (CDC). The two most commonly identified causative agents of foodborne salmonellosis are S. enterica serovars Typhimurium and Enteritidis. Both serovars have the ability to colonise the reproductive organs of hens (the oviduct and ovary) and are major causes of foodborne illness. Globally, Salmonella Enteritidis is more commonly linked to contaminated eggs, except in Australia, where the majority of egg-related foodborne salmonellosis is caused by Salmonella Typhimurium*”. These data, and those above, suggest that *Salmonella* Typhimurium has the potential to pose a health threat like that of *Salmonella* Enteritidis, although global health surveillance data indicate that it is on a much lower scale. This will be addressed below, to an extent. In the UK a large proportion of chickens are vaccinated against both *Salmonella* Enteritidis and *Salmonella* Typhimurium under the Lion Scheme.
- 7.8 *Salmonella* Typhimurium of various phage types (DTs) predominates in laying flocks and eggs in Australia and is responsible for egg-borne food poisoning outbreaks. Some of this may be related to farm hygiene problems and high ambient temperatures, resulting in stress for the birds, but the ability of Australian *Salmonella* Typhimurium strains to cause vertical contamination of egg contents is not known.
- 7.9 *Salmonella* Typhimurium is associated with eggs to a lesser extent than *Salmonella* Enteritidis in Europe (EFSA and ECDC, 2013), but is the serovar mostly associated with laying hens and eggs in a small number of other geographical areas worldwide (Jamshidi *et al.*, 2010; Singh *et al.*, 2010; Chousalkar and Roberts, 2012). In France, for example, *Salmonella* Typhimurium 4, 5, 12 (aphasic, non-motile) has been recently linked to a foodborne outbreak related to the consumption of tiramisu (Le Hello *et al.*, 2012). *Salmonella* Typhimurium strains can be further subdivided into definitive phage types (DTs), according to their susceptibility to a series of bacteriophages (Anderson *et al.*, 1977; Rabsch *et al.*, 2002). Some *Salmonella* Typhimurium DTs (such as DT104 and DT49) are able to infect a broad range of animal species, while others are host adapted (such as DT2, DT40 and DT99

in wild birds and DT8 in ducks) (Rabsch *et al.*, 2002). When *Salmonella* Typhimurium types that are host adapted to other avian species infect chickens, they normally cause a short-lived infection (Martelli and Davies, 2012). Other *Salmonella* Typhimurium DTs, such as DT104 and DT49, can infect chickens and cause egg contamination (Threlfall *et al.*, 1990; Williams *et al.*, 1998; Okamura *et al.*, 2010), although this has mostly been demonstrated in artificial infection studies. The virulence and invasiveness of *Salmonella* Typhimurium is also determined by the bacterial strain (Barrow *et al.*, 1987; Keller *et al.*, 1997; Okamura *et al.*, 2010; Wales and Davies, 2011). For example, an artificial infection study investigating the invasiveness and egg contamination potential of 10 *Salmonella* Typhimurium DT104 strains showed that they differed in their ability to cause ovarian infection and egg contamination (Okamura *et al.*, 2010).

- 7.10 Other *Salmonella* serovars (e.g. *Salmonella* Senftenberg, *Salmonella* Livingstone, *Salmonella* Infantis) are occasionally isolated from eggs, mainly from eggshells, but also rarely from egg contents (Martelli and Davies, 2012). *Salmonella* Infantis has been isolated from eggs in several surveys carried out in Europe (de Louvois, 1993; Little *et al.*, 2007a; Murchie *et al.*, 2007; Wilson, 2007; Chemaly *et al.*, 2009; Martelli and Davies, 2012). *Salmonella* Infantis is also associated with eggs and human illness in geographical areas outside Europe, such as Japan and New Zealand (Lapuz *et al.*, 2008; Wilson, 2007).
- 7.11 Experimental infection using high-dose challenge has demonstrated that certain strains of *Salmonella* Hadar, *Salmonella* Infantis, *Salmonella* Montevideo and *Salmonella* Heidelberg could contaminate the interior of eggs after high dose intravenous or intravaginal challenge. In recent years a multi-drug resistant strain of *S. Heidelberg* has emerged in the USA and Canada and has resulted in numerous egg-borne *Salmonella* food poisoning outbreaks as well as becoming established in broiler production through vertical transmission and hatchery contamination. *Salmonella* Heidelberg appears to be more resistant to the biocidal action of egg albumen than *Salmonella* Virchow or *Salmonella* Hadar. *Salmonella* Heidelberg was also commonly found in the ovaries of spent hens in earlier surveys, but *Salmonella* Agona, *Salmonella* Oranienberg, *Salmonella* Mbandaka, *Salmonella* Kentucky, *Salmonella* Montevideo, and *Salmonella* London were all found more commonly than *Salmonella* Enteritidis at that time (late 1980s). Variability in the ability of different *Salmonella* serovars to penetrate intact egg shells and multiply in egg contents has also been shown, but most experimental models were not really representative of levels or modes of contamination that would be likely to occur in commercial egg production. Lublin *et al* (2008) using artificial contamination experiments found that *Salmonella* Virchow behaved essentially the same as *Salmonella*

Enteritidis on egg shells and in egg yolks. Later work by these authors Lublin *et al* (2015) also found that *Salmonella* Infantis survived better on egg shells at refrigeration temperatures. In yolk at low temperatures, following a small initial decline, numbers remain stable. There was extensive growth in yolk in eggs at room temperature. *Salmonella* Infantis can also penetrate egg shells.

***Salmonella* Enteritidis has a greater ability to infect reproductive hen tissues and contaminate egg contents.**

- 7.12 This has been addressed in some detail in Chapter 3 but the importance of showing that *Salmonella* Enteritidis has different behaviours in hens and their reproductive tracts from other *Salmonella* demands that the issue is also examined in this section. De Vylder *et al* (2013) showed that *Salmonella* Enteritidis survived significantly better in eggs than other serovars including *Salmonella* Typhimurium, other members of Serogroup B, Serogroup D, excluding *Salmonella* Enteritidis, and Serogroup E and G isolates. In a review article, Perry and Yousef (2012) provided a detailed examination of the behaviour of *Salmonella* Enteritidis in laying hens. They quote the work of a variety of others. In one *in vitro* study of egg follicles, *Salmonella* Enteritidis adhered at higher levels than *Salmonella* Typhimurium. *Salmonella* Enteritidis also attached to vaginal epithelial cells in greater numbers compared to other serovars including *Salmonella* Typhimurium, Agona, and Heidelberg. It is suggested that the type of lipopolysaccharide (LPS) produced by a given serovar may play a role in its interaction with hen tissues. A study (Parker *et al* 2001) comparing LPS O-chains of *Salmonella* Enteritidis and *Salmonella* Typhimurium supports this suggestion. This work analysed several strains from both serovars and found that *Salmonella* Enteritidis strains were much more likely to produce an O-chain of high molecular mass and egg isolates were likely to produce glycosylated O-chains. The authors theorised that members of the *Salmonella* Enteritidis serovar may be uniquely capable of altering the characteristics of the O-chain produced depending on their environment. Wales and Davies (2011), in a review paper, concluded that *Salmonella* Enteritidis adhered better to reproductive tissues and was more frequently found in egg contents. However, *Salmonella* Typhimurium invoked more pathology in reproductive tissues and a more intense immune response which is likely to result in clearance rather than persistence of infection.
- 7.13 Recent UK research funded by BBSRC and FSA also found that the structure of the bacterial LPS in the outer membrane played the major role in reproductive tract infection and in survival in egg albumen, particularly at hen body temperature (Coward *et al.* 2012 and 2013). For example *Salmonella* Typhimurium survives more poorly than *Salmonella* Enteritidis in egg albumen

at hen body temperature (42°C) but better at 25°C. There are also differences between *Salmonella* Enteritidis phage types at hen body temperature.

- 7.14 Several researchers have investigated the colonisation of avian reproductive tracts after artificial inoculation with various *Salmonella* serovars. Keller *et al* (1997) found that the frequency of colonisation of the reproductive tract to be similar between *Salmonella* Enteritidis and *Salmonella* Typhimurium but found only *Salmonella* Enteritidis in forming eggs. This is consistent with UK data showing that *Salmonella* Enteritidis survives better than *Salmonella* Typhimurium in egg albumen at hen body temperature (Coward *et al.* 2012 and 2013). In other experiments, Okamura *et al.* (2001) inoculated mature hens intravenously with six different *Salmonella*. All were able to colonize reproductive tracts but *Salmonella* Enteritidis did so at significantly higher levels and was the only serovar recovered from laid eggs. In another study conducted by the same group, hens were inoculated intravaginally. Similar results were observed with regard to reproductive tissues, and all serovars were found associated with eggshells, but only *Salmonella* Enteritidis and *Salmonella* Typhimurium were isolated from egg contents (Okamura *et al.*, 2001). In more recent studies by Gast *et al* (2011), hens were inoculated orally with *Salmonella* serovars including *Salmonella* Enteritidis, Heidelberg, and Hadar. No differences in numbers of *Salmonella* in ovaries or oviducts were seen. However, the rate of internal egg contamination was significantly higher (3.58%) in chickens infected with *Salmonella* Enteritidis than the other strains (0.47% and 0%), respectively. While caution should always be exercised in the interpretation of studies where artificial infection is used, they do support data indicating that *Salmonella* Enteritidis is the serovar most often found in association with eggs and egg-containing foods. This poses the hypothesis that there are some fundamental differences between *Salmonella* Enteritidis and other *Salmonella*, which allows it to not only better infect hen reproductive tissues and contaminate eggs *in vivo*, but also to survive better in the forming egg. Recent UK work (Coward *et al* 2012 and 2013) supports the view of Parker *et al* (2001) that LPS structure is key to the abilities described above.
- 7.15 Many genes have been identified, in a number of *Salmonella*, to have roles in colonisation of avian tissues and organs and contamination or survival in chicken eggs. Genes unique to *Salmonella* Enteritidis or strains of it will be discussed below.
- 7.16 Lu *et al* (2003) identified the gene *yiz.fD* in *Salmonella* Enteritidis which, when transformed into *Salmonella* Typhimurium, significantly increased transformant survival in albumen at 37°C. This gene is involved in repair of damage to DNA, which is likely to be caused by exposure to the toxic environment of egg albumen (Clavijo *et al*, 2006) used transposon mutagenesis to identify

additional genes important for the survival of *Salmonella* Enteritidis in egg albumen. The authors identified several mutants with increased susceptibility to albumen, the majority of which carried insertions in sequences related to cell wall structure and function or amino acid metabolism. One gene sequence absent from *Salmonella* Typhimurium was also identified and confirmed to play a role in survival. From this work, it was concluded that cell wall integrity is a main contributor to bacterial survival in albumen and that *Salmonella* Enteritidis possess certain genetic variations that make it more suited than other serovars to survive in the environment of the egg (Clavijo *et al* 2006). A recent study cited the importance of genes involved in the synthesis of O antigens (Gantois *et al* 2009). The authors observed increased transcription of this gene in cells incubated in albumen at room temperature. A knockout mutant was unable to grow under identical conditions or to survive in albumen at higher temperatures. A mechanism to explain these observations was not proposed, but the findings support the suggestion of Mizumoto *et al.* (2005) who showed that altered *Salmonella* Enteritidis LPS plays a role in egg contamination. Raspoet *et al* (2014b) used transposon mutagenesis to reveal that LPS structure and the heat shock protein HtrA were essential for the survival of *Salmonella* Enteritidis in egg albumen at hen body temperature.

- 7.17 UK work referred to in Chapter 3 (Coward *et al*, 2012 and 2013) and work in the US confirmed the importance of LPS structure in infection of the hen reproductive tract. Even fine differences in LPS structure can have a profound difference in the behaviour of *Salmonella* Enteritidis and *Salmonella* Typhimurium and variants of these in the hen reproductive tract and the forming egg, although such effects have not been examined in detail with other *Salmonella* serovars. One important feature is that the albumen antibacterial agent lysozyme adheres much less effectively to the LPS of *Salmonella* Enteritidis than that of *Salmonella* Typhimurium at hen body temperature although there was little difference in adherence at room temperature (Coward *et al* 2012 and 2013). In other UK experiments where the LPS of *Salmonella* Typhimurium was replaced by that of *Salmonella* Enteritidis, *Salmonella* Typhimurium survived significantly better at hen body temperature.
- 7.18 The genetic homogeneity of *Salmonella* Enteritidis strains has been confirmed by several researchers (Botteldoorn *et al.*, 2010; Porwollik *et al.*, 2005). Despite the high level of genetic relatedness revealed by these studies, differences have been observed among the phenotypes of *Salmonella* Enteritidis isolates, especially in relation to invasiveness (Yim *et al.*, 2010). Researchers have also noted that small genetic differences may correlate closely to PTs (Betancor *et al.*, 2009). Guard *et al* (2010) showed that *Salmonella* Enteritidis sub-populations vary in their abilities to infect chicken reproductive tissue and contaminate eggs. In one recent study a small

polymorphism within the *rpoS* gene, which encodes a stress response regulator, was directly tied to decreased invasiveness and reduced survival in egg albumen (Shah *et al.*, 2012).

- 7.19 It is clear from surveillance, outbreak investigation and studies of hens using artificial infection that there are *Salmonella* serovars that have the potential to pose a threat to public health. However, it is our view that the threat is at a much lower level than that which was posed by *Salmonella* Enteritidis and is largely confined to areas of the world outside the UK.

Conclusions

- 7.20 *Salmonella* Typhimurium is associated with eggs to a lesser extent than *Salmonella* Enteritidis in Europe (EFSA and ECDC, 2013), but is the serovar mostly associated with laying hens and eggs in a small number of other geographical areas worldwide
- 7.21 *Salmonella* Infantis is also associated with eggs and human illness in geographical areas outside Europe, such as Japan and New Zealand
- 7.22 When comparing *Salmonella* Enteritidis and Typhimurium, the former has been shown to adhere better to reproductive tissues and is more frequently found in egg contents. However, *Salmonella* Typhimurium has been shown to invoke more pathology in reproductive tissues and a more intense immune response which is likely to result in clearance rather than persistence of infection.
- 7.23 It has been shown that *Salmonella* Enteritidis survives better than *Salmonella* Typhimurium in egg albumen at hen body temperature
- 7.24 It is clear from surveillance, outbreak investigation and studies of hens using artificial infection that there are *Salmonella* serovars that have the potential to pose a threat to public health. However, it is our view that the threat is at a much lower level than that which was posed by *Salmonella* Enteritidis and is largely confined to areas of the world outside the UK.

Recommendation

- 7.25 Information should be obtained on characteristics associated with the ability to vertically transmit into the egg contents so that new and emerging *Salmonella* strains with these characteristics can be identified at an early stage.

Chapter 8: Importance of surveillance and identification of emerging threats

Surveillance considerations

- 8.1 The sensitivity of identification of human *Salmonella* infection varies dramatically between countries (Felicio *et al.*, 2015) and is influenced by surveillance and reporting policies and economics. In many countries only severe human cases of salmonellosis are reported and this makes the apparent hospitalisation and mortality rates appear disproportionately high in these countries. Detection methodology may vary and often relatively insensitive methods are used for clinical cases in an attempt to simultaneously identify *Salmonella* and *Shigella*. These methods are suitable for clinical investigations but may under-estimate the occurrence of intermittent or low-level shedders.
- 8.2 Another influencing factor is the prominence of *Salmonella* in the media. If recent outbreaks have been featured, more people are likely to seek medical attention and a sample is more likely to be taken; becoming ill after an egg-based meal is also likely to stimulate further inquiries.
- 8.3 The very low prevalence of *Salmonella* contamination of eggs, and particularly of contents, means that effective surveillance of eggs is not considered to be economically feasible, but there may be periodic or targeted surveys, particularly aimed at high-risk sources such as imported eggs from certain countries when there is a known or suspected *Salmonella* problem in egg production. A more sensitive way of detecting contamination issues would be to sample egg packing facilities after a day's work but before any routine cleaning. Eggs passing through the system are rolled, shaken and brushed as part of the grading process and this releases surface contaminants which accumulate as fine dust on and beneath packing equipment. This can be readily sampled with a moist fabric swab, which is ideally collected directly into pre-enrichment broth on site. If positive samples are found in a packing plant further investigations can be carried out, but a series of well-taken negative samples is a good indication that eggs entering the plant are not likely to be contaminated (Davies and Breslin, 2003).
- 8.4 Another problem with egg tests is the inhibitory nature of the egg albumen and deficiency in available iron, such that the sensitivity for detection of *Salmonella* from egg contents is low. Addition of iron sources such as Ferrioxamine E can substantially increase the detection of *Salmonella* from egg contents, particularly pooled eggs, but the egg must also be uncontaminated by other *Enterobacteriaceae* as additional iron can preferentially stimulate growth of

competing organisms, thus reducing the chance of detecting *Salmonella*. The same problem applies to the extended pre-enrichment time that is often applied to egg contents, which can also lead to overgrowth of non-target organisms if the egg contents are not collected aseptically.

- 8.5 Disinfection of egg surfaces before sampling contents may also affect the isolation rate. Immersion in boiling water or alcohol may affect *Salmonella* that is located only in shell membranes rather than in shell contents, but if surface decontamination is not done before breaking the egg, there is a chance of contamination of egg contents during sampling. It is desirable to test the whole eggshell rather than just swabbing the surface, so a compromise method is to separate contents from shells without any disinfection and test shells and contents separately. If there is a positive result with the same strain on both shells and in contents, the contents result would be described as “suspect” rather than positive in subsequent analyses (Carrique-Mas & Davies, 2008).
- 8.6 The sensitivity of surveillance of laying hen flocks has been discussed above and is low. Only one sample is tested on each sampling occasion and the maximum test sensitivity for identification of *Salmonella* if it is present in the sample is 85%. The major problem is non-uniform distribution of *Salmonella* in a laying house so the sample may or may not contain *Salmonella*, or the level of *Salmonella* compared to other *Enterobacteriaceae* in a pooled sample is so low that it is not possible to prevent overgrowth of non-target organisms, resulting in a false-negative test. It is physically difficult to obtain a representative sample in large poultry houses, particularly colony cage houses where manure belts beneath cages cannot be accessed to obtain a sample so the final single pooled sample, e.g. from a house containing over 100,000 birds, only represents a fraction of the birds and even if sampling was perfect, each bird would only contribute 0.03g of faeces to the pool, which is further reduced when a 25g subsample is taken from the pool of 300g for testing. Dust is a much more representative sample as there are focal points such as egg elevators and exhaust vents where naturally mixed samples can easily be taken and isolation of *Salmonella* from dust is easier than from faeces (Carrique-Mas *et al.*, 2008). The egg industry however objects to using dust as a sample on the grounds that birds may not really be infected, but in a laying house this is unlikely, and it has been shown that if *Salmonella Enteritidis* is found in dust it can also be found in post-mortem samples from birds, but it may take several hundred birds before the infection is confirmed. Sampling every 15 weeks during lay using an insensitive method results in an average of 4-5 samples tested during the life of a flock. Most positive samples come from flocks at the end of lay but infection usually occurs immediately after introducing birds into the laying houses. The detection of infection can be

further reduced by treatment with antibiotics or organic acids or addition of lime to the litter of non-cage flocks before sampling with boot swabs. As most of the sampling is carried out by the producers themselves, there is also an opportunity for deliberate deception, normally by supplying samples from known negative birds to the laboratory or pressure cooking the sample.

- 8.7 With the emergence of new epidemic strains of *Salmonella* within serovars such as Infantis, Kentucky, Stanley, it is important to apply sensitive monitoring and to share data so that incursion of such strains into UK can be dealt with at an early stage whilst it is still possible to do so. The UK poultry industry has faced incidents of *Salmonella* Paratyphi B variant Java, multidrug resistant *Salmonella* Infantis and highly ciprofloxacin resistant *Salmonella* Kentucky, as identified by NCP monitoring programmes, and these have all been stamped out before they could become permanently established and disseminate further.

Molecular Epidemiology

- 8.8 With improved control of *Salmonella* in British laying flocks the pattern of *Salmonella* Enteritidis infection in people has changed substantially. Not only has there been an overall reduction in cases but also the PTs that are found increasingly reflect sources outside the UK, for both travel-related and “domestically” acquired cases. A combination of phage typing and antimicrobial resistance testing has been particularly useful to elucidate the origin of particular strains, e.g. nalidixic acid resistance is characteristic of isolates from those countries where fluoroquinolone antibiotics are inexpensive and have been routinely used in previous years, or in some cases currently, to control *Salmonella* in breeding flocks. This particularly applies to certain Mediterranean and Eastern European Countries. Comparison of PTs between countries is becoming increasingly difficult, however, since in many countries no phage typing is carried out at all, and in others it is only done for human or animal isolates, but not both, and is rarely done for isolates from foodstuffs. In recent years some countries have discontinued phage typing and even the EU Reference Laboratory for *Salmonella* no longer conducts phage typing, and European ring trials to ensure consistency between phage-typing laboratories are to be discontinued. PHE at Colindale now routinely uses Whole Genome Sequencing (WGS) as a replacement for serotyping, for *Salmonella* Enteritidis. Such data will be optimised by inclusion in a national and international surveillance infrastructure to monitor trends, outbreaks and sources/reservoirs/pathways.
- 8.9 EFSA and ECDC have led the introduction of Pulsed Field Gel Electrophoresis (PFGE) – based typing of *Salmonella* and provided databases and analytical

capacity, but there is no mechanism for funding individual EU Member States to carry out this laborious method of typing and it is not suitably sensitive to differentiate within *Salmonella* Enteritidis, which is mostly highly clonal as a result of its rapid emergence and dissemination through the poultry breeding pyramid.

- 8.10 Other sensitive typing methods such as CRISPR typing have emerged but are only done in a limited number of laboratories. Multiplex PCR-based SNP-based typing has been developed by identifying key targets by whole genome sequencing (WGS). WGS itself offers the best medium and long-term solution however since it can simultaneously confirm isolates as *Salmonella* identify the species or subspecies, serotype, identify antimicrobial resistance genes present, detect virulence genes including those thought to be involved in vertical transmission and survival in eggs and also differentiate live vaccine strains from field strains (EFSA, 2013). It is therefore possible to replace the battery of existing tests that are carried out on *Salmonella* with WGS, and although the method is currently relatively expensive and requires considerable investment in equipment and skilled personal, the costs continually reduce. The epidemiological precision offered by WGS for outbreak investigation and attribution will be much more valuable than systems that are currently in place. It should also be possible to analyse the combinations of genes that are associated with factors such as egg invasion, virulence and epidemic potential so that newly emerged strains with these undesirable characteristics can be identified in food animals at an early stage and stamped out before further dissemination makes the cost of doing this prohibitive. It is to be hoped that in the future EFSA and ECDC will promote the use of WGS, facilitate data storage and analysis and that EC will provide co-funding for EU Member States to develop and apply this methodology to *Salmonella* surveillance programmes in all sample types from humans, the food chain, companion animals and the environment.
- 8.11 It nevertheless remains a concern that analytical activities applied to WGS data will for some considerable time, not have available the considerable archive of data from human, animal and foodstuffs analysed using serotyping and phage typing which provide a background to events both current and in the future. Whole genome sequencing remains a state of the art method with much still to be uncovered and understood.

Conclusions

- 8.12 With the emergence of new epidemic strains of *Salmonella* within serovars such as Infantis, Kentucky, Stanley, it is important to apply sensitive monitoring

and to share data so that incursion of such strains into UK can be dealt with at an early stage whilst it is still possible to do so.

- 8.13 It is to be hoped that in the future EFSA and ECDC will promote the use of WGS, facilitate data storage and analysis and that EC will provide co-funding for EU Member States to develop and apply this methodology to *Salmonella* surveillance programmes in all sample types from humans, the food chain, companion animals and the environment.

Chapter 9: Recommendations

Key recommendation

The Group recommends that the Food Standards Agency considers amending its advice on eggs in the light of the Group's risk assessment.

Other recommendations by chapter

Chapter 1

- 1.87 We recommend that the Agency and other Government Departments continue to monitor UK egg outbreaks associated with *Salmonella* Enteritidis and other microbiological hazards and ensure that the Committee is updated regularly to ensure that our advice is reviewed and updated as necessary.
- 1.88 We acknowledge the significant efforts undertaken by the UK egg industry since the 1990s to reduce the prevalence of *Salmonella* Enteritidis in laying hen flocks which in turn has made a remarkable impact in reducing the level of human *Salmonella* Enteritidis infections in humans.
- 1.89 We recommend that at timely intervals and with resources permitting, that regular surveys are carried out to assess the level of *Salmonella* contamination of hens' eggs on retail sale and used in catering establishments in the UK and that the origins of any contaminated eggs are recorded. We would like to be kept informed of the outcomes of any surveys.
- 1.90 We recommend that further data are gathered on sales of other types of eggs including duck and quails eggs and if possible we recommend that further data are gathered at regular intervals to assess the contamination levels of such eggs.
- 1.91 We recommend that measures to improve the traceability of egg supplies, especially those within the catering sector, be considered.

Chapter 3

- 3.18 We recommend that data relating to internet sales of different types of eggs are gathered by the most suitable means to determine the extent to which internet sales influence the UK egg market.

Chapter 4

- 4.31 We recommend reinforcement of good hygiene guidance and training in all settings.
- 4.32 We recommend that up-to-date information relating to catering practices, such as pooling and storage of eggs, is obtained.
- 4.33 We recommend that trends in the duck egg market are monitored and consideration given to exploring the extension of existing NCP regulation to ducks. As part of this we also recommend monitoring of *Salmonella* occurrence in these eggs to be better placed to assess the public health risk.
- 4.34 FSA advice remains that eggs should be stored in the refrigerator below 8°C in catering, food production and domestic premises.
- 4.35 Consumer preferences for unprocessed catering/home-prepared food containing raw eggs, along with deliberate undercooking of such foods increases the risk posed by *Salmonella* with eggs not sourced from schemes with a comprehensive suite of control measures like the UK Lion Code, or schemes equivalent to it. Eggs from any source should be protected from cross-contamination by any potential food poisoning bacteria. The Working Group supports FSA advice that if caterers do not purchase eggs produced under the Lion Code or a scheme equivalent to it, they should use pasteurised egg for any food which is likely to be served uncooked, or lightly cooked.
- 4.36 Two studies in the catering industry (2002, 2006) have identified poor practice in relation to egg storage and handling, including pooling of eggs, in catering. FSA should review the uptake/impact of the most recent FSA advice in this area (2014).
- 4.37 FSA should ensure that those involved in the storage, handling and use of eggs know that the risks in relation to *Salmonella* in eggs are significantly affected by egg sources and history, specifically, the risks associated with eggs produced and distributed within an appropriate comprehensive quality and safety management system such as that provided by the British Lion mark certified farm assurance scheme are likely to be lower than the risks associated with eggs which are not produced in, and protected by, such systems.

Chapter 5

- 5.50 Robust, evidence based methods for the validation of the performance of heat-processing treatments are probably required because current methods may overestimate the microbial load reduction achieved, with industrial procedures for heat treatment of egg products.

Chapter 6

6.23 It would be practical, and advantageous, to bring together the FSA exposure assessment model with updated data from UK surveillance and with the modified Australian model, proposed by the EFSA BIOHAZ panel, to form an improved assessment for hazards that are associated with *Salmonella* in eggs in the UK.

Chapter 7

7.25 Information should be obtained on characteristics associated with the ability to vertically transmit into the egg contents so that new and emerging *Salmonella* strains with these characteristics can be identified at an early stage.

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Chapter 2

11.5 We recommend that the government promotes the extension of adequate surveillance systems in Europe (para 2.26).

The data collection on human diseases from Member States is now according to Decision 1082/2013/EU on serious cross-border threats to health. Since 2004, the establishment of the European Centre for Disease Control (ECDC) and 2002 with the establishment of the European Food safety Authority, new systems are in place for reporting Member State national surveillance data in humans (TESSy) and animals, feed and food (EFSA Data Collection Framework under Directive 2003/99/EC).

11.6 We further recommend that approaches be made to other national authorities to assess the potential for harmonising the output from current or future surveillance systems (para 2.27).

Since 2001, improvements have been made to analysis and reporting of zoonoses at the EU level as well as collaborative working on systems for outbreak detection and management of cross border threats (SOPs for ECDC, EFSA and Member States). There are no specific harmonised surveillance requirements for human zoonoses but there are defined case definitions for infectious disease reporting in Decision 2012/506/EU. There are some harmonised monitoring requirements and harmonisation of case definitions plus legal reporting obligations for animals, feed and food under Directive 2003/99/EC which also covers the production of the annual European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks covering zoonoses in humans, animals, feed and food as well as antimicrobial resistance. Recent initiatives for further data sharing to facilitate joint response to cross border threats – the EFSA/ECDC molecular database (although unlikely to enable participation by England due to different typing methods used ie whole genome sequencing and fla typing etc) and the Expert Opinion on the introduction of next-generation typing methods for food- and waterborne diseases in the EU and EEA.

(http://ecdc.europa.eu/en/publications/_layouts/forms/Publication_DispForm.aspx?List=4f55ad51-4aed-4d32-b960-af70113dbb90&ID=1389)

11.7 We recommend that the Food Standards Agency (FSA) takes such steps as necessary to ensure that it receives regular reports on foodborne disease from the UK's surveillance systems and, if possible, from Enternet (para 2.28).

'Enternet' does not exist anymore as it has been subsumed by the ECDC system TESSy. The FSA does receive such reports from the Department of Health as part of the monitoring process for foodborne diseases. The Agency also chairs regular meetings of the cross Government Epidemiology of Foodborne Infections group (EFIG) where information relating to foodborne disease is readily shared between members and with the ACMSF.

11.8 We recommend that the FSA keeps under regular review the need to commission targeted epidemiological studies to explore or expand the output from the routine surveillance systems of foodborne disease (para 2.29).

The Agency considers data on trends in *Salmonella* infections on a regular basis and formally through the EFIG which provides twice yearly updates to the Agency and ACMSF. PHE has recently introduced whole genome sequencing for *Salmonella* isolates submitted to the reference laboratory for the Gastrointestinal Bacteria Reference Unit; these data potentially provide greater opportunities for more timely detection and investigation of *Salmonella* outbreaks.

Chapter 3

11.14 We recommend that the FSA, in collaboration with other relevant bodies, reviews the existing Government guidance on outbreak investigation and, in due course, issues an updated version which takes account of our concerns (para 3.29).

The FSA has produced a revised version of guidance on outbreak investigations in 2008.

<https://www.food.gov.uk/business-industry/guidancenotes/hygguid/outbreakmanagement>

Additional communicable disease outbreak management plans have also been published by Public Health England and their equivalents in the devolved nations for [England](#), [Wales](#), [Northern Ireland](#) and [Scotland](#).

11.15 We recommend that the FSA requests relevant statutory agencies to report promptly to it any *Salmonella* outbreak which appears likely to be associated with the consumption of eggs in some form or other. We further recommend that the FSA then uses its powers to ensure that all necessary investigations are carried out, at whatever level is appropriate, in a coordinated and expeditious manner (para 3.30).

The documents referred to in the response to 11.14 include guidance on the investigations to be undertaken during the management of outbreaks and can be applied to *Salmonella* outbreaks that are likely to be associated with eggs.

The FSA regularly updates Food Law Codes of Practice and Food Law Practice Guidance, Links to the documents updated in 2015 are as follows:

- Food Law Codes of Practice 2015 sets out responsibilities of the enforcement authorities, including notifying of food incidents;
<http://fsahome/Pages/default.aspx>
- The FSA also issues associated Food Law Practice Guidance to assist the competent authorities in enforcing the Food Law. Chapters 7.5 and 7.5 cover specifically eggs.
<http://www.food.gov.uk/sites/default/files/Food%20Law%20Practice%20Guidance%20October%202015%20-%20FINAL%20.pdf>
- The Incident Management Plan (IMP), which can be found the link below, outlines our plans and procedures for meeting our responsibilities in response to non-routine food-related incidents
<http://www.food.gov.uk/sites/default/files/FSA%20Incident%20Management%20Plan.pdf>

11.16 We recommend that the FSA, in conjunction with other relevant agencies, reviews the systems in place in the UK for the surveillance of foodborne infections in order to assure itself that appropriate data are being collected and reported in a timely and meaningful way (para 3.31).

The Agency chairs the EFIG group. The group meets twice yearly and serves as a platform for the surveillance and monitoring of foodborne infections in the UK relating to *Salmonella* and other gastrointestinal (GI) pathogens. Trends and comments by surveillance bodies at EFIG meetings are incorporated into papers presented to ACMSF.

Chapter 6

11.29 We recommend that the Government, preferably in conjunction with industry, sets up a monitoring procedure for the prevalence of *Salmonella* in commercial laying flocks so that trends of infection can be followed (para 6.55).

This recommendation has been met by the setting up of the *Salmonella* National Control Programmes (NCPs) in the poultry sectors. The layer NCP commenced on 1st January 2009 across the EU, and requires regular sampling of laying flocks. It also makes it illegal to supply table eggs from flocks testing positive for *Salmonella* Typhimurium or *S. Enteritidis* into the food chain without heat treatment to kill salmonella. The EU legislation also requires the reporting of all isolations of salmonella, and this is further backed up by the Zoonoses Order 1989, which makes isolations of *Salmonella* reportable to Defra. The resulting data are analysed and reported to the EU annually.

11.30 We recommend that the level of infection in the commercial egg laying sector is established, to inform a risk assessment, before any changes are considered to the UK policy on slaughter or the need for egg pasteurisation (para 6.56).

(See above. It should also be noted that pasteurisation of eggs from flocks testing positive from *S. Typhimurium* or *S. Enteritidis* is now a legal requirement).

11.31 We recommend that sampling carried out for Government-funded, supported or approved research or surveillance should not be subject to the normal reporting arrangements required under the Zoonoses Order (para 6.57).

This is still an issue not in so much that reporting is carried out, but that the new EU legislation (see above) potentially requires egg pasteurisation in the event of a positive *S. Typhimurium* or *S. Enteritidis* result. This could be addressed by requiring re-testing of positive flocks in research programmes with the standard EU test and taking no action should that test be negative.

11.32 We recommend that the Government should introduce arrangements which would enable it to monitor the quality of existing arrangements for the monitoring of feed and feed ingredients at feed mills (para 6.58). We believe that such monitoring could make a positive contribution to the early detection of emerging, potentially pathogenic *Salmonella* serotypes. It is thus important for Government to ensure that it works efficiently and that any deficiencies are remedied.

In 2009, the '*Code of Practice for the Control of Salmonella during the Production, Storage and Transport of Compound Feeds, Premixtures, Feed Materials and Feed Additives*' was published by Defra, with significant input from the Agency's Animal Feed Branch.

The Code was a result of a collaborative effort between several government departments and agencies, the National Farmers Union and representative organisations across the food, feed and agriculture sectors. The Code was also discussed and subsequently endorsed by the Advisory Committee on Animal Feedingstuffs.

The aim of the Code was to provide non-statutory guidelines to ensure that compound feedingstuffs, premixtures, feed materials and additives are of a satisfactory bacteriological quality, and to minimise the risk of *Salmonella* contamination.

The Defra Code of Practice can be found here:

<http://www.food.gov.uk/sites/default/files/multimedia/pdfs/committee/copsal.pdf>

11.33 We recommend that the FSA seeks to ensure that available surveillance data, from whatever source, are utilised as an early warning system to detect the emergence of *Salmonella* strains possessing the potential for invasiveness of chickens' reproductive systems (para 6.59).

This recommendation has been addressed in that data from the now extensive official surveillance programmes (NCPs) are widely and promptly shared by Defra with other government departments, including FSA, through (for example) the EFIG group. Such an incident would rapidly be brought to the attention of the FSA by APHA / Defra.

11.34 We recommend that the FSA encourages discussions within the European Union about the creation of a surveillance system designed to monitor *Salmonella* infection in egg production systems (para 6.60).

This recommendation has been addressed (see answers above).

Chapter 7

11.45 We recommend that the work undertaken by the Veterinary Laboratories Agency to assess the effectiveness of vaccination under field conditions of extreme environmental exposure to *Salmonella* should be completed as soon as possible (para 7.41).

A series of projects that included field studies on the introduction of, and changes to, vaccination programmes as well as in-vivo challenge studies were carried out. These demonstrated the benefit of vaccination, but vaccinal protection can be overwhelmed by high level challenge, e.g. via infected breeding mouse populations in poultry houses or undermined by poor vaccination administration techniques. A holistic approach to maximise the benefit of vaccination has been promoted within the egg industry.

11.46 We recommend immediate Government-funded surveillance to assess whether the overall level of contamination in UK hens' eggs has reduced since the 1995/96 survey, including a comparison between eggs from vaccinated flocks and eggs from flocks where control measures do not include vaccination. If it is also possible to incorporate differentiation between cage produced eggs and the main

alternative – the free range production system – this could further enhance the survey. If not, great care should be taken to reflect the weighting of the various production systems in the sampling protocol (para 7.42).

The Agency has commissioned a number of egg surveys to investigate contamination levels associated with a range of different eggs types.

- The FSA carried out a survey of *Salmonella* contamination of UK-produced shell eggs on retail sale in response to a recommendation by the Committee in its 2001 report (Food Standards Agency, 2004). The survey was carried out between March and July 2003. A total of 4753 samples (mostly boxes) of six eggs were purchased from a representative cross-section of retail outlets throughout the UK and the shell and contents tested for *Salmonella* contamination. The overall finding was that nine samples (0.34%) were contaminated with *Salmonella*, which was equivalent to 1 in 290 “boxes” of 6 eggs. All *Salmonella* positive samples were from egg shells only. Comparison with the 1995/96 survey indicated that there had been a threefold reduction in the prevalence of *Salmonella* (from 0.99% to 0.34%). However, the most common *Salmonella* serovar isolated was still *Salmonella* Enteritidis. There was no statistically significant difference between the prevalence of *Salmonella* contamination in samples purchased in England, Scotland, Wales or N. Ireland; or between the prevalence of *Salmonella* contamination in samples from different egg production types or between non-Lion code eggs and Lion code eggs or between eggs that were stored chilled or at ambient temperature, but the statistical power of the study was low because of the small number of isolates. However, there was a statistically significant higher prevalence of *Salmonella* contamination of eggs from medium sized retailers than large ones.

<http://tna.europarchive.org/20110116113217/http://www.food.gov.uk/science/surveillance/fsis2004branch/fsis5004eggs>

- FSA (2006). Survey of *Salmonella* Contamination of Non-UK Produced Shell Eggs on Retail Sale in the North West of England and London. The FSA commissioned a survey of *Salmonella* contamination of non-UK eggs on retail sale in London and the North West of England over a period of 16 months, between March 2005 and July 2006. The estimated prevalence of all *Salmonella* and *Salmonella* Enteritidis was reported to be 3.3% and 2.6%, respectively.
- The Agency carried out a survey of *Salmonella* contamination of raw shell eggs used in catering premises between November 2005 and January 2007.

A total of 1,588 pooled samples of six eggs were collected at random from 1,567 catering premises in England, Wales, Scotland and Northern Ireland.

The overall finding was that six pooled samples were found to be contaminated with *Salmonella* on the shell of the egg giving a prevalence of 0.38%. Two different serotypes were recovered of which the most common was *Salmonella* Enteritidis (5/6). There were three different phage types of this serovar with PT4 predominating (3/5). *S. Mbandaka* was also isolated. *Salmonella* was detected from five egg samples comprising eggs that were UK produced and one from eggs produced in Germany. The survey's kitchen practice element showed evidence of poor egg storage and handling practices in catering premises (Food Standards Agency, 2007).

11.47 If the results of the surveillance recommended in paragraph 7.42 confirm the industry's findings that eggs are rarely contaminated with any *Salmonella* spp. then we further recommend that the environmental work undertaken by the Veterinary Laboratories Agency be extended to explore more fully the reasons for the apparent virtual disappearance of all *Salmonella* serovars from egg production (para 7.43).

At the request of the Agency, the ACMSF has established an *Ad Hoc* group on eggs in 2015 to assess the current level of risk from shell eggs and their products and to assess how the risks relating to *Salmonella* may have changed since 2001 when the Committee last examined the subject in detail. As part of this report, the epidemiology of *Salmonella* serovars are explored in detail.

11.48 We recommend that the FSA explores with key stakeholders the means by which the wider use of vaccination can be promoted (para 7.45).

Vaccination to protect against *Salmonella* Enteritidis is currently virtually universal in all commercial scale laying hen flocks, and also in many backyard flocks as it is not easy to purchase non-vaccinated pullets. Vaccination against *S. Typhimurium* is also used for most Lion Code flocks and independent free range flocks.

11.49 In addition, we recommend that the FSA takes all necessary steps to satisfy itself that no unnecessary impediments are being placed in the way of the development and licensing of new vaccines (para 7.45).

Salmonella vaccines continue to be developed and authorised. Currently there are seven *Salmonella* vaccines authorised for use in the UK of which 4 of these (AviPro *Salmonella* Duo, AviPro *Salmonella* Vac T, Gallimune Se + St and Gallimune Se) have been authorised since 2001. Vaccines currently authorised for use in the UK have been approved following a harmonised EU authorisation procedure (mutually

recognised) except on one occasion where the vaccine, AviPro Salmonella Vac E, was nationally authorised in 2000.

11.50 In relation to Northern Ireland, we recommend that the FSA there investigates the circumstances of the recent fall in human *Salmonella* cases in order to elucidate the reasons for this reduction and the possible contribution made by the increased availability of eggs from *S. enteritidis*-vaccinated flocks (para 7.46).

Although N. Ireland has not been specifically investigated, it is evident that for the UK as a whole, *Salmonella* infection rates have fallen dramatically and are comparable across the UK.

Chapter 8

11.62 We believe that “Use by” dates on eggs would be of more value to consumers than current markings and we recommend that the Government takes this point up in the appropriate EU forum (para 8.35).

Requirements for date marking of eggs are set out in the European Egg Marketing Regulations (Regulation (EC) 589/2008). The European Commission is considering egg marketing standards as part of a wider review of marketing standards across agricultural sectors. The Government is working closely with the Commission to consider all proposals under the review.

11.63 We recommend that, subject to competing priorities, the Government should consider commissioning research into how consumers handle eggs in the domestic environment (para 8.36).

No specific research in this area has been undertaken, but the Agency’s Food and You survey does address this by exploring methods used by consumers to determine whether foods including eggs are safe to eat. The survey reports in percentage terms, behaviours used by consumers to determine egg safety. The latest relevant report is here:

<http://www.food.gov.uk/sites/default/files/food-and-you-2014-uk-bulletin-2.pdf>

11.64 We recommend that, in developing its communications strategy, the FSA should keep in mind our concerns about the possible risks from raw shell eggs (para 8.37).

The FSA’s advice to consumers has since the 1990s been reflective of the Committee’s concerns of the possible risk from raw shell eggs; the advice was also available through NHS choices. The Agency is aware that the epidemiology relating to *Salmonella* Enteritidis outbreaks linked to hens’ eggs in the UK has changed over the years and has now sought the Committee’s advice to determine the current level

of microbiological risk of shell eggs and egg products, a decade on to ensure that the Agency's advice takes account of the best available up to date information.

11.65 We also recommend that the FSA draws to the attention of the relevant trade associations our concerns about the safety of dishes where raw eggs are both bulked and served without any cooking (para 8.38).

The egg leaflet issued in 2002 to caterers included advice to use pasteurised egg for raw or lightly cooked foods. The advice reissued in 2014 added: "if you are breaking eggs to use later (sometimes called 'pooling') keep the liquid egg in the fridge and take out small amounts as needed. Use all 'pooled' liquid egg on the same day and don't add new eggs to top it up."

11.66 We reiterate the recommendations in our first Report that caterers should continue to increase their use of pasteurised egg, particularly for dishes that are not subject to further cooking prior to consumption, and that manufacturers, retailers and consumer organisations should consider how best to encourage consumers to use pasteurised egg instead of shell egg, where appropriate (para 8.39).

In 2002, the FSA distributed a leaflet to caterers which gave advice on storage, handling, the use pasteurised egg for raw or lightly cooked foods, especially for vulnerable groups, and not to use eggs after the "best before" date.

The Agency advised caterers to use pasteurised egg for all foods that would not be cooked or would be only lightly cooked, and recommended that the safest option for caterers preparing food for more vulnerable people was to use pasteurised egg for all foods, even those that are cooked.

In 2014 the FSA published on its website a reminder to caterers of its advice on the safe handling of eggs: See <http://www.food.gov.uk/news-updates/news/2014/12842/fsa-advice-to-caterers-on-the-safe-handling-of-eggs#sthash.ua0emZH2.dpuf>

In 2002, advice on the FSA's Consumer website "Eatwell" included information on storage, cleaning, avoiding cross-contamination, and not using eggs after the "Best before" date. It also included the following:

If you're preparing food for elderly people, babies, toddlers, pregnant women or people who are already unwell, you shouldn't use raw egg in any food that won't be cooked. You could use pasteurised egg instead (available from some supermarkets), because pasteurisation kills bacteria.

When you're eating out, or buying food that isn't labelled, and you're not sure whether a food contains raw egg, ask the person serving you.

11.67 We recommend that our concerns about the possible role of environmental contamination in the causation of outbreaks is brought to the attention of both industry and enforcement authorities (para 8.40).

- Existing FSA advice relating to eggs and food safety considers cross-contamination and environmental contamination. For example, the Agency advises that eggs are kept away from other foods, both when they are in the shell and after you have cracked them.
- Be careful not to splash egg onto other foods, worktops or dishes.
- Always wash and dry your hands thoroughly after touching eggs or working with them.
- Clean surfaces, dishes and utensils thoroughly, using warm soapy water, after working with eggs.
- Don't use eggs with damaged shells, because dirt or bacteria might have got inside them.

11.68 We recommend that, as a matter of urgency, the existing research into the relationship between knowledge about food safety and possession of the basic and intermediate food hygiene certificates be repeated. If the original findings are confirmed, then we further recommend that the bodies that award these certificates re-evaluate their examination processes (para 8.41).

The Agency has addressed this recommendation through its ongoing work with Improve Ltd, the Sector Skills Council for the UK's Food and Drink Manufacturing and Processing sector, to support development of national occupational standards and content for food safety qualifications that provide an understanding of the risks to food safety and how to control these

11.69 We recommend that, subject to competing priorities, consideration be given to commissioning research into consumers' attitudes towards post-production treatments of eggs aimed at increasing the margin of safety from *Salmonella* infection (para 8.42).

The main source of information on attitudes/ eggs is from the Agency's Food and You survey which includes a question on eggs/ and methods for checking whether they are safe to eat, use-by and best before dates are included in these methods. The latest relevant report is here:

<http://www.food.gov.uk/sites/default/files/food-and-you-2014-uk-bulletin-2.pdf>.

11.70 Again, subject to competing priorities, we recommend that consideration is given to commissioning research into whether changes to traditional recipes may increase the risk of food poisoning from certain dishes (para 8.43).

The Agency has reviewed this recommendation and considers that the current climate is somewhat different to that when the Committee's last report was published. Our advice over the years has been clear to highlight any risks associated with shell eggs and their products, and provide examples of higher risk foods as part of our advice.

Chapter 9

11.72 In relation to research we recommend that, subject to competing priorities and against the background of their existing programmes, Government and other funding bodies should consider the case for future research in the following areas:

- the factors which influence the growth of *Salmonella* in egg contents, the survival of *Salmonella* on-shell and *Salmonella* transferability (paras 9.2-9.3);
- the contaminated farm environment (para 9.4);
- aerosols (para 9.5);
- contamination of eggs in egg packing plants (paras 9.6-9.8);
- virulence and pathogenicity (paras 9.9-9.11);
- detection and differentiation of *Salmonella* strains (para 9.12);
- egg washing (para 9.13);
- reduction and elimination of *S. enteritidis* (para 9.14); and
- consumer behaviour (paras 9.15-9.18).

ACMSF papers ACM 678 and ACM 880 provide further details. The Agency has funded a programme of four projects. The programme began in 2000. ACM/678 provides details of the projects which are:

- A study to examine the egg-to-egg variations in the growth of *Salmonella* spp. in egg contents.
- Cross contamination from the external surface of eggs in relation to risk of exposure to *Salmonella*.
- A review of commercial egg washing with particular emphasis on the control of *Salmonella*
- Pilot study to estimate the nature and extent of adherence to government guidance on safe egg use in the catering industry

11.73 Our recommendations in relation to surveillance appear in Chapter 2 (paragraphs 2.26 *et seq*), 3 (paragraph 3.31), 6 (paragraph 6.55 *et seq*) and 7 (paragraph 7.42).

Chapter 10

11.77 We recommend that, if further work on risk assessment is required, the necessary empirical research should be encouraged or, if necessary, commissioned (para 10.20). Once the results of such research are available, it should be possible to use and develop the prototype models produced so far on a realistic basis, to provide a quantitative risk assessment covering different scenarios for egg production, distribution and food preparation.

11.78 In the meantime, it is also recommended that:

- the FSA should make the risk assessment model freely available, whilst emphasising its provisional status (and the purely illustrative nature of any current results);
- users be encouraged to use the model with alternative inputs and to feed back to the FSA their views on its actual and potential usefulness; and
- in the light of such feedback, the FSA considers commissioning further work to make the model more user-friendly in order to encourage its wider use (para 10.21).

Since 2001 more information has become available particularly on *Salmonella* in laying flocks, prevalence of *Salmonella* contamination in UK and non UK eggs. The Food Standards Agency has used some of these data to populate and further develop the model as well as running a workshop with experts to obtain their opinion to help bridge key data gaps. The FSA has developed the DH deterministic model further into a probabilistic model using Monte Carlo simulation to model uncertainty. The model estimates the prevalence of *Salmonella* contamination at each step in the food chain from farm to fork by compounding probabilities of cross contamination at each stage. This provides for two key outputs:

- Probability of eggs being contaminated at point of sale
- Probability of an egg based meal/dish being contaminated at point of consumption.

The model draws on a range of data sources including FSA egg survey results, and findings from DEFRA research projects with remaining data gaps having been addressed using expert opinion. The predicted prevalence of contaminated eggs at point of sale has been validated against findings from the Food Standard Agency's

2003 UK retail egg survey. The model has also been peer reviewed by the Veterinary Laboratories Agency Centre for Epidemiology & Risk Analysis.

ANNEX II

Details on surveys of *Salmonella* contamination in table eggs considered in Martelli *et al.*, unpublished. Information on the localisation of the isolates is provided when available (S: shell only; C: contents only; S+C: both shells and contents). Where available, the origin of imported eggs is detailed in a footnote.

Country (year) and references	Positives/samples (pool size)	Shell only	Contents only	Both contents and shell	SE	ST	SO	SO list
UK (Jan 1991-Dec 1991) (de Louvois, 1993) 1993 British eggs.	65\7045 (pools of 6 eggs)	48	7	10	47 (30S, 7C, 10S+C)	6	12	<i>S. Infantis</i> (1), <i>S. Livingstone</i> (8), Others not specified (3).
UK (Jan 1991-Dec 1991) (de Louvois, 1993) 1993 Imported eggs (a).	138\8630 (pools of 6 eggs)	110	2	26	19 (14S, 2C, 3S+C)	9	110	<i>S. Infantis</i> (55), <i>S. Livingstone</i> (31), <i>S. Braenderup</i> (8), Others not specified (16).
UK (Jan-Feb1991), British eggs. CVL Weybridge Unpublished data	18\2510 (pools of 6 eggs)	13	3	2	8 (6S, 2S+C)	3 (2S, 1S+C)	7	<i>S. Livingstone</i> (2S), <i>S. Derby</i> (1S), <i>S. Isangi</i> (1S), <i>S. Untypable</i> (1S), <i>S. Senftenberg</i> (2C).
UK (June1991-July1992) Eggs packed in England and Wales (MAFF CVL Weybridge, Unpublished data)	122 (pools of 6 eggs)	97	14	11	65 (51S, 6C, 8S+C)	7 (7S)	50	<i>S. Virchow</i> PT26 (6S, 1S+C), <i>S. Livingstone</i> (incomplete data), <i>S. Goldcoast</i> (4S+3C), 0:Z:1,6 (1S+1C), <i>S. Agama</i> (2S, 1S+C, 1C), <i>S. Panama</i> (4S), <i>S. Braenderup</i> (3S), <i>S. Poona</i> (2C, 1S+C), Untypable (incomplete report), <i>S. Bredeney</i> (1S), <i>S. Derby</i> (1S), <i>S. Heidelberg</i> (1S), <i>S. Newport</i> (incomplete report), 4,12:-:1 (1S).
UK (1995-1996) (ACMSF, 2001) UK eggs.	138\13970 * (pools of 6 eggs)	NA	NA	NA	119	6	19	<i>S. Mbandaka</i> (4), <i>S. Livingstone</i> (5), <i>S. Kimuenza</i> (2), <i>S. Indiana</i> (2), <i>S. Virchow</i> (2), <i>S. Infantis</i> (1), <i>S. Braenderup</i> (1), Other serotypes (2).
UK (1996-1997) (ACMSF, 2001) Imported eggs.	29\1433 (pools of 6 eggs)	NA	NA	NA	18	0	11	<i>S. Taksony</i> (5), <i>S. Livingstone</i> (2), <i>S. Braenderup</i> (2), <i>S. Virchow</i> PT2 (1), <i>S. Infantis</i> (1).

UK (2002) London catering establishments (Little, Surman-Lee, <i>et al.</i> , 2007)	7\726 (pools of 6 eggs)	NA	NA	NA	at least 2	0	at least 2	<i>S. Cerro</i> , <i>S. Livingstone</i> .
UK 2002-2004 Catering England and Wales associated with SE outbreaks (b). (Little, Surman-Lee, <i>et al.</i> , 2007)	88\2102 * (pools of 6 eggs)	NA	NA	NA	80	0	33	<i>S. Infantis</i> (12), <i>S. Livingstone</i> (2), <i>S. Altona</i> (7), <i>S. Bredeney</i> (1), <i>S. Ohio</i> (11).
UK (2003) Catering eggs (c) (Elson, Little, & Mitchell, 2005)	17\5686 (pools of 6 eggs)	NA	NA	NA	15	1	1	<i>S. Livingstone</i> (1).
UK(2003) UK produced shell eggs on retail sale (FSA, 2004)	9\4753 (pools of 6 eggs)	9	0	0	7 (7S)	0	2	<i>S. Infantis</i> (1S), <i>S. Livingstone</i> (1S).
UK (2004) Eggs from positive flocks (Davies & Breslin, 2004)	92\13652 (pools of 6 eggs)	78\13652	9\13640	5\13682	33\13682 (24S, 6C, 3S+C)	2\13652 (2S)	57\13682	<i>S. Infantis</i> (41S+2C), <i>S. Livingstone</i> (11S), <i>S. Newport</i> (2).
UK (2005 - 2006) Imported eggs (d) (FSA, 2007; Little, Walsh, <i>et al.</i> , 2007)	157\1744 * (pools of 6 eggs)	147	NA	10	136 (129S, 7S+C)	0	21	<i>S. Braenderup</i> (1S), <i>S. Infantis</i> (1S), <i>S. Mbandaka</i> (11S+ 3C), <i>S. Panama</i> (1S), <i>S. Rissen</i> (2S), <i>S.</i> <i>Unnamed</i> (6S), <i>S. Weltevreden</i> (1S).
UK (2006) Catering premises (f) (FSA, 2007; Little <i>et al.</i> , 2008)	6\1588 (pools of 6 eggs)	5	NA	1	5 (4S, 1 S+C)	0	1	<i>S. Mbandaka</i> (1S).
UK (2008) Catering establishments (Little <i>et al.</i> , 2008)	1\764 (mixed size pools)	NA	NA	NA	1	0	0	NA
NORTHERN IRELAND (1996-7) (I. G. Wilson, Heaney, & Powell, 1998)	9\2090 (pools of 6 eggs)	8	1	0	39 (2S, 1C)	1(1S)	5	<i>S. Mbandaka</i> (1S), <i>S. Montevideo</i> (1S), <i>S. Infantis</i> (2S), <i>S. Kentucky</i> (1S).

REPUBLIC OF IRELAND (2003) (Anonymous, 1993)	0\1169 (pools of 6 eggs)	NA	0	NA	NA	NA	NA	NA
IRELAND (2005-2006) (Murchie <i>et al.</i> , 2007)	2\5018 (pools of 6 eggs)	2	0	0	0	0	2	<i>S. Infantis</i> (1S), <i>S. Mondevideo</i> (1S).
ALBANIA (1996-1997) Imported eggs (g) (Telo, Bijo, Sulaj, & Beli, 1999)	1\79 (pools of 10 eggs)	1	0	0	0	0	1	<i>Salmonella</i> group C (no further serotyped).
FRANCE (2008) Eggs collected from positive flocks (Chemaly <i>et al.</i> , 2009)	44\4200 (individual eggs)	44	NA	NA	17 (17S)	3 (3S)	24	<i>S. Montevideo</i> (2S), <i>S. Virchow</i> (18S), <i>S. Infantis</i> (4S).
JAPAN (2007-2008) Catering eggs (Sasaki <i>et al.</i> , 2010)	5\2030 * (pools of 10 eggs)	5	0	0	2 (2S)	0	3	<i>S. Derby</i> (2S), <i>S. Livingstone</i> (1S), <i>S. Cerro</i> (1S).
JAPAN (2004-2006) Soiled eggs (dirty) (Lapuz <i>et al.</i> , 2008)	30\1766 (pools of 90 eggs)	NA	NA	30	7 (S+C)	1? (S+C)	22/23	<i>S. Infantis</i> (22), 1 no data available.
JAPAN (2004-2006) Processed eggs (clean) (Lapuz <i>et al.</i> , 2008)	116\11280 (pools of 40 eggs)	NA	116	NA	112 (C)	0	4	<i>S. Infantis</i> (4C).
JAPAN (2004-2006) Packed eggs (supermarket) (Lapuz <i>et al.</i> , 2008)	3\9010 (pools of 10 eggs)	NA	3	NA	2 (C)	0	1	<i>S. Infantis</i> (1C).
URUGUAY (2000-2002) (Betancor <i>et al.</i> , 2010)	58\620 (pools of 20 eggs)	NA	58	NA	8 (C)	0	50	<i>S. Derby</i> (39C), <i>S. Panama</i> (2C), <i>S. Gallinarum</i> (9C).

USA- ARKANSAS (1994) (Schutze, Fawcett, Lewno, Flick, & Kirby, 1996)	1\100 (pools of 12 eggs)	1	0	0	0	0	1	<i>S. Heidelberg</i> (1S).
USA (1993-1994) Eggs at washing plants (1) (F. T. Jones, Rives, & Carey, 1995)	8\180 (individual samples)	8	NA	NA	0	0	8	<i>S. Heidelberg</i> and <i>S. Montevideo</i> .
USA (1993-1994) Eggs at washing plants (2) (F. T. Jones <i>et al.</i> , 1995)	0\180 (individual samples)	NA	0	NA	0	0	0	NA
USA (2006) Restricted eggs (D. R. Jones & Musgrove, 2007)	2\180 (pools of 6 eggs)	1	0	0	0	0	2	<i>S. Heidelberg</i> (2S).
HAWAII (1989) (Ching-Lee, Katz, Sasaki, & Minette, 1991)	10\106 * (pools of 12 eggs)	10\106	0	0	0	0	10	<i>S. Braenderup</i> (2S), <i>S. Oranienburg</i> (4S), <i>S. Mbandaka</i> (1S), <i>S. Ohio</i> (1S), <i>S. Havana</i> (1S), <i>S. Montevideo</i> (2S), <i>S. Livingstone</i> (1S).
CANADA (1996) Eggs from washing and grading stations. (Poppe, Duncan, & Mazzocco, 1998)	1\252	NA	NA	1	0	0	1	<i>S. Agona</i> (1).
NEW ZEALAND (2005-2006) (Wilson, 2007)	9\514 (pools of mixed sizes)	9\514	0	0	0	0	9	<i>S. Infantis</i> (9S).
AUSTRALIA (2009) (Chousalkar, Flynn, Sutherland, Roberts, & Cheetham, 2010)	0\500 (individual eggs)	0\500	0	0	0	0	0	NA

SOUTH INDIA (1997-1998) (Suresh, Hatha, Sreenivasan, Sangeetha, & Lashmanaperumalsamy, 2006)	39\492 * (individual eggs)	30	0	9	35 (26S, 9S+C)	0	4	<i>S. Cerro</i> (2S), <i>S. Molade</i> (1S), <i>S. Mbandaka</i> (1S).
NORTH INDIA (2006-2007) Eggs from poultry farms (Singh, Yadav, Singh, & Barthy, 2010)	10\260 (individual eggs)	2	7	1	0	9 (9S)	1	<i>S. Africana</i> (1).
NORTH INDIA (2006-2007) Eggs from marketing channels (Singh <i>et al.</i> , 2010)	17\300 (individual eggs)	10	5	2	0	6 (S)	11	<i>S. Lagos</i> (6), <i>S. Rough Strain</i> (4), <i>S II</i> (1).
IRAN (June-August 2008) Retail outlets (Jamshidi, Kalidari, & Hedayati, 2010)	4\250 (individual eggs)	4	0	0	0	4 (S)	0	NA

SE: *Salmonella* Enteritidis

ST: *Salmonella* Typhimurium

SO: *Salmonella* serovars other than SE or ST

NA: Not available

* More than one serovar was found in one or more samples.

Countries of origin of the eggs and number of pools of eggs analysed for each country:

(a) Belgium (550), Denmark (830), France (350), Germany (750), The Netherlands (6130), Italy (20).

(b) UK (528), Germany (2), Portugal (50), USA (60), Spain (1100) and Not Known (362).

(c) UK (4987), Spain (22), Germany (10), Portugal (7), Republic of Ireland (3), Holland (3), Italy (3), Not Known (651).

(d) Belgium (13), France (348), Germany (45), Poland (4), Portugal (25), Republic of Ireland (23), Spain (1157), The Netherlands (129).

(f) UK (1413), Spain (48), Germany (38), The Netherlands (33), France (27), Portugal (8), Republic of Ireland (1), Poland (1), Mixed origin UK and Spain (2), Not Known (17).

(g) Bulgaria (60), Italy (6), Greece (6), Turkey (2), Rumania (2) Macedonia (2), Hungary (1).

Legislative Requirements – EU legislation

Food Hygiene Rules Relating to Eggs and Egg products

Regulation (EC) No. 852/2004 lays down the basic food hygiene requirements for all food businesses. Annex I of the regulation provides requirements for primary production food business activities (e.g. farming and growing) and Annex II lays down requirements for premises, equipment, training and hygiene of food handlers, provisions for foodstuffs etc.

Regulation (EC) No. 853/2004 lays down specific hygiene rules for food of animal origin. Section X, Chapter I of the regulation lays down requirements for egg and egg products, storage, transportation temperatures and maximum time limit for when eggs must be delivered to the consumer.

Regulation (EC) No. 854/2004 lays down specific rules for the organisation of official controls on products of animal origin intended for human consumption

Regulation (EC) No. 2073/2005 lays down microbiological criteria for certain micro-organisms and the implementing rules to be complied with by food business operators. The Regulation lays down food safety criteria for *Salmonella* in egg products which defines the acceptability of foodstuffs placed on the market.

Regulation (EC) No. 882/2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. The Regulation requires that official controls carried out by the MSs must enable them to verify and ensure compliance with rules on feed and food. To this end, official controls must in principle be carried out at any stage of production, processing and distribution of feed and food. These controls are defined based on the identified risks, the experience and knowledge gained from previous controls, the reliability of the controls already carried out by the business operators concerned, and a suspicion of possible non-compliance.

Regulation (EC) No. 2160/2003 on the control of *Salmonella* and other specified foodborne zoonotic agents lays down a framework for the reduction of zoonoses and zoonotic agents in animals at all relevant stages of the food chain, but principally at the farm level to reduce the risk to public health. Currently the controls are aimed only at *Salmonella* in specified poultry populations: chickens and turkeys. The Regulation requires various defined measures to be put in place in all Member States including the implementation of harmonised *Salmonella* National Control Programmes (NCPs) to monitor the progress towards achieving a set agreed reduction target, defined for each poultry sector in the implementing Regulations.

Regulation (EC) No. 1177/2006 implementing Regulation (EC) No 2160/2003 as regards requirements for the use of specific control methods within the framework of the national programmes for control of *Salmonella* in poultry. This Regulation prohibits the use of antimicrobials as a specific method to control *Salmonella* in poultry except under specific defined circumstances. Vaccination is permitted for the control of *Salmonella* as long as an authorised vaccine is used and vaccine strain can be differentiated from wild/field strain *Salmonellae*. Additionally the Regulation specifies that vaccination must be used if the EU baseline survey results indicated >10% prevalence for the target serovars in a Member State, although derogations are available.

Regulation (EC) No 1237/2007 amending Regulation (EC) No 2160/2003 and Decision 2006/696/EC as regards the placing on the market of eggs from *Salmonella* infected flocks of laying hens provides for measures to restrict the placing on the market of table/Class A eggs and for an additional enhanced sampling protocol where there may be suspicion of false positive results detected during the control programme sampling. The marketing restrictions defined are:

- from 1 November 2007 in any case where the flock is identified as the source of a *Salmonella* outbreak in humans (any *Salmonella* spp)
- from 1 January 2009 if the flock is detected positive for *S. Enteritidis* or *S. Typhimurium*

Regulation (EC) No. 200/2010 implementing Regulation (EC) No 2160/2003 as regards a Union target for the reduction of the prevalence of *Salmonella* serotypes in adult breeding flocks of *Gallus gallus* (amending Regulation 1003/2005). This Regulation specifies the target for reduction to 1% or less flocks detected positive for 5 regulated serovars: *S. Enteritidis*, *S. Typhimurium*, *S. Hadar*, *S. Infantis* and *S. Virchow*. The Regulation requires operators to take samples from each breeding flock every two to three during hatching egg production and for official samples on two or three occasions during the life of the flock. The aim of the legislation is to prevent spread of infection to production poultry/down food chain via hatching eggs

Regulation (EC) No. 517/2011 implementing Regulation (EC) No 2160/2003 as regards a Union target for the reduction of the prevalence of certain *Salmonella* serotypes in laying hens of *Gallus gallus* and amending Regulation (EC) No 2160/2003 and Commission Regulation (EU) No 200/2010. The target specified is an annual % reduction from baseline or for a reduction to 2% or less flocks detected positive for *S. Enteritidis* and *S. Typhimurium*. The Regulation requires producers to monitor their flocks for *Salmonella* every 15 weeks during egg production and official sampling is required once annually in one flock on all premises with 1000 or more birds. The aim of the legislation is to optimise detection of infection, allowing the placing of egg marketing restrictions in the event a regulated serovar is detected.

Additional Legislation Relating to Eggs and Egg Products

Directive 2003/99/EC regarding monitoring of zoonoses and zoonotic agents requires EU Member States (MSs) to collect, evaluate and report data on zoonoses, zoonotic agents and antimicrobial resistance in food, animals and animal feedstuffs and foodborne outbreaks to the European Commission (EC) each year. The system is based on current surveillance systems in place in the MSs, with some data monitoring harmonised under EU legislation. (Data collection on zoonotic human diseases from MSs is conducted in accordance with Decision 1082/2013/EU).

Regulation (EC) No. 798/2008 - provides a list of Third Countries approved for imports of eggs in shell for human consumption in the EU. This legislation enables the implementation of the legal requirement for imports of poultry and poultry products from Third Countries to comply with the same public health risk reduction requirements as those in place in Member States – i.e. in the implementation of equivalent *Salmonella* control programmes under Regulation (EC) No. 2160/2003.

Regulation (EC) No 183/2005 laying down requirements for feed hygiene including the general implementation of procedures based on the principles of hazard analysis and critical control points (HACCP), together with the application of good hygiene practice for preventing or limiting *Salmonella* contamination during transport, storage and processing of feed materials.

Regulation (EU) No. 142/2011 implementing Regulation (EC) No 1069/2009 laying down health rules as regards animal by-products and derived products not intended for human consumption. This legislation requires that processed animal proteins must comply with the *Salmonella* criterion laid down in the Regulation

Legislative Requirements – UK legislation

The EU legislation is implemented through the following national legislation:

- Control of Salmonella in Poultry (England) Order 2007 (SI 2007/3574) and equivalent legislation in the Devolved Governments provides the legislative basis for the National Control Programme in breeding flocks of *Gallus gallus* and laying hens producing eggs for human consumption.
- Egg and Chick Regulations (2009) England (SI 2009/2163) and equivalent in the Devolved Governments, covers production through to final sale, laying down stamping, labelling and permitted marketing terms for eggs. The Eggs and Chicks Regulations in England and Wales also lay down *Salmonella* control related requirements (NB Scotland and Northern Ireland do not have these provisions in their devolved legislation). These provisions provide financial penalties for operators who contravene or fail to comply with Regulation (EC) 2160/2003. The EU regulatory provisions which apply to the

marketing of eggs for consumption are contained in Council Regulation (EC) No. 1308/2013 and Commission Regulation (EC) No. 589/2008. These regulations cover the quality and weight grading, marking, packaging, storage, transport, labelling for retail sale.

- Registration of Establishments (Laying Hens) (England) Regulations 2003 and equivalent legislation in the Devolved Governments provides for registration of establishments keeping laying hens (sites with more than 350 birds) and provision of this information to public health authorities where this is necessary to trace eggs put on the market for human consumption.
- The Food Safety and Hygiene (England) Regulations 2013, the regulations consolidate the General Food Regulations 2004 and the Food Hygiene (England) Regulations 2006 into one piece of legislation (for England only).
- The Food Information Regulations 2014 (FIR) are the domestic regulations that establish the enforcement measures for Regulation (EU) No 1169/2011 on the provision of food information to consumers (EU FIC).

Glossary

APHA	Animal and Plant Health Agency. An executive agency of the Department for Environment, Food and Rural Affairs.
Attenuated vaccine	A vaccine created by altering the virulence of a pathogen so that it becomes harmless.
Auxotrophic	The inability of an organism to synthesize a particular organic compound required for its growth.
Bacteriophage	A virus whose host is a bacterium
Bacteriostatic	The ability to inhibit the growth of some types of bacteria
Biofilm	A film of microorganisms
Ciprofloxacin	An antibiotic used to treat a number of bacterial infections.
ECDC	European Centre for Disease Prevention and Control
ELISA	Enzyme-Linked Immunosorbent Assay. A laboratory test for specific antibodies.
Epithelial	Pertaining to or involving the outer layer of the skin
Hydrocolloid	A substance that forms a gel with water used to thicken or stabilize food products.
Immuno-compromised	Used to describe someone who has an impaired immune system, usually due to treatment or underlying illness
Leucosis/sarcoma group	A group of viruses that cause transmissible diseases in poultry.
Laid in Britain and Lion code	
LPO antigen	
LPS	Lipopolysaccharide
Lumen	The opening in a hollow organ
Lysozyme	An enzyme occurring in, amongst other things, egg white that can kill bacteria
Macrophages	
Monophasic	Having a single phase.
Mycoplasma	
Nalidixic acid	An antibacterial drug
Non-typhoidal Salmonella (NTS)	
Oocyte	A cell from which an egg develops
Ovotransferrin	A protein in egg white
Rappaport-Vassiliadis Medium (RVM)	A medium used for the enrichment of salmonellae.
Reticuloendotheliosis	A retrovirus infection of chickens, turkeys, ducks, geese, and quail with morbidity up to 25%.

Polymorphonuclear leucocytes	White blood cells having a lobed nucleus
Prevalence	The number of instances of disease or event in a given population at a specific time.
Psychrotolerant	Tolerant to cold
Psychrotrophic spoilage organisms	Organisms that can grow at low temperatures.
PFGE	Pulsed-field gel electrophoresis. A technique used to separate DNA fragments by size.
PT4, PT8 etc and DT	PT = Phage type. DT= Definitive phage type. Phage typing is a method used to distinguish between bacteria within the same species.
Pullet	A young female chicken
Salmonella	Gram-negative rod shaped bacteria
Serovar	A subdivision within a species of bacteria
Transposon mutagenesis	
Virulence	The capacity of an organism to cause disease
VLA	Veterinary Laboratories Agency
WGS	Whole genome sequencing. A laboratory process that determines the whole DNA sequence of an organism's genome at a single time.
Zoonotic pathogen	Organism able to cause disease/illness in an animal that is transmissible to humans.

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