

18/03/04

**REPORT OF THE SURVEY OF *SALMONELLA*  
CONTAMINATION OF UK PRODUCED SHELL  
EGGS ON RETAIL SALE**

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## ABBREVIATIONS

ACMSF	Advisory Committee on the Microbiological Safety of Food
BEIC	British Egg Industry Council
BGA	Brilliant green agar
BPW	Buffered peptone water
CI	Confidence Intervals
CSL	Central Science Laboratory
DARD NI	Department of Agriculture and Rural Development in Northern Ireland
DEFRA	Department for Environment Food and Rural Affairs
DH	Department of Health
DLS	Direct Laboratories Services
EMI	Egg Marketing Inspectorate
EQA	External Quality Assurance
FEPAS <sup>®</sup>	Food Examination Performance Assessment Scheme
FSA	Food Standards Agency
GLM	Generalised Linear Model
HACCP	Hazard Analysis of Critical Control Points
HPA	Health Protection Agency (formerly PHLS)
LACORS	Local Authority Co-ordinators of Regulatory Affairs
LEP	Laboratory of Enteric Pathogens
LIMS	Laboratory information management systems
NMR	National Milk Records
PFGE	Pulsed field gel electrophoresis
PHLS	Public Health Laboratory Service
PT	Phage Type
QM	Quality Management Ltd
QUB	Queen's University Belfast
RVS	Rappaport-Vassiliadis soya peptone broth
SEERAD	Scottish Executive Environment and Rural Affairs Department
SOP	Standard Operating Procedure
UKAS	United Kingdom Accreditation Service
UKEP	United Kingdom Egg Producers
XLD	Xylose lysine desoxycholate agar

## EXECUTIVE SUMMARY

The FSA's survey of *Salmonella* contamination of UK-produced shell eggs on retail sale was carried out over a period of 5 months, between March and July 2003. The main objective was to establish the prevalence of *Salmonella* contamination in these eggs and whether this had changed since the previous retail survey conducted in England in 1995/96.

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. In terms of different production types of eggs 50.0% were from caged production, 16.9% were from free-range systems, 16.6% were from organic systems and 16.5% were from barn systems.

The overall UK finding was that 9 samples (0.34%) were contaminated with *Salmonella*, which is equivalent to approximately 1 in every 290 "boxes" of 6 eggs.

In the last major survey, conducted in 1995/96, the eggs were sampled in England only. On this occasion eggs were sampled from all four countries in the UK. If the findings from the current survey are compared on an England only basis then there has been a 3-fold reduction in the level of *Salmonella* contamination since 1995/96 and this is likely to reflect the measures introduced by the UK egg industry to control *Salmonella*.

Factors that might have influenced whether or not eggs were contaminated with *Salmonella* were also examined. However, where differences were found these tended to be small and much larger sample sizes would have been required to demonstrate a statistically significant difference. There was no statistically significant difference between the prevalence of *Salmonella* contamination in samples purchased in England, Scotland, Wales or Northern 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. However, there was a statistically significant higher prevalence of *Salmonella* contamination of eggs from medium sized retailers\* than large retail outlets.

Of the 9 isolates from *Salmonella* positive samples 7 (78%) were *S. Enteritidis* and of these, 3 were *S. Enteritidis* phage type 4 (PT4). There were also single isolates of *S. Infantis* and *S. Livingstone*. All of the *Salmonella* isolates were fully sensitive to 10 antimicrobial agents and none of the 3 *S. Enteritidis* PT4 isolates corresponded to known vaccine strains. *Salmonella* *Infantis*, *S. Livingstone* and *S. Enteritidis* PTs 4, 6 and 12 were found in previous egg surveys.

In addition to the 9 *Salmonella* positive samples there were a further 5 egg samples which were reported as positive for *S. Dublin*. This was an unusual and unexpected finding 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 *S. Dublin* findings, it is most likely to have resulted from cross-contamination during the handling and testing of eggs. The Agency considers that there is sufficient doubt about the validity of the *S. Dublin* findings to justify excluding them from the main analysis. The interpretation of the main findings from the statistical analysis remain the same with or without the inclusion of the *S. Dublin* findings.

All *Salmonella* positive samples were from egg shells. Comparison with the 1995/96 survey indicated that the prevalence of *S. Enteritidis* PT4, which is most commonly associated with eggs and human illness, in samples of 6 eggs have fallen sharply from 0.58% of samples to 0.11% in 2003.

It is not unusual for *Salmonella* to be present in the environment and therefore not surprising that a few isolates were found from egg shells. The small number of positive samples points towards random contamination from the production environment rather than any systemic contamination from infected flocks.

\*independent/local shops

## **ACKNOWLEDGEMENTS**

The Food Standards Agency would like to thank all organisations, companies and individuals that contributed to the planning, execution and reporting of this survey. We would particularly like to mention the contractors – Direct Laboratories, Charis Food Research (part of the Scottish Agricultural College), Queen’s University Belfast, the Health Protection Agency’s Laboratory of Enteric Pathogens and Central Science Laboratories, for the external quality assurance tests used to ensure accurate *Salmonella* analyses from our contractors. We would also like to thank the Egg Marketing Inspectorate at Defra for providing us with information and advice during the survey and all retailers/producers of shell eggs that agreed to take part in the survey, for their co-operation in the collection of samples.

## 1. INTRODUCTION

**1.1** Human salmonellosis is a significant cause of morbidity, mortality and economic loss (Roberts and Sockett 1994; Adak *et al.* 2002; Roberts *et al.* 2003). Between 1981-1991 the number of cases of salmonellosis in humans in the UK rose by approximately 170% and remained high throughout most of the 1990s. In March 1991, the Advisory Committee on the Microbiological Safety of Food (ACMSF) agreed to set up a working group to consider the extent to which eggs were responsible for this problem. Their report was published in 1993 and the Committee concluded that much of the rise in human salmonellosis was due to *Salmonella* Enteritidis, mostly phage type 4 (PT4), which can invade the reproductive tract of chickens (ACMSF 1993). The Committee's work was informed by a Department of Health (DH) funded survey undertaken between February and November 1991 to assess the prevalence of *Salmonella* contamination of UK-produced eggs from high street retail outlets in England and Wales. This involved testing 7045 groups of 6 eggs of which 0.92% were found to be contaminated with *Salmonella* with an estimated contamination rate per individual egg of 1 in 650 eggs on either the shell and/or in the contents (de Louvois, 1993). *Salmonella* Enteritidis and *S. Enteritidis* PT4 comprised 72% and 51% of the *Salmonella* isolates respectively.

**1.2** A further DH funded retail survey of UK-produced eggs in England was undertaken between May 1995 and April 1996. *Salmonella* was detected in 0.99% of 13970 samples of 6 eggs giving an estimated contamination rate per individual egg of 1 in every 100 boxes (ACMSF 1993). A survey of retail eggs in Northern Ireland between April 1996 and October 1997 found *Salmonella* in 0.43% of 2090 samples of 6 eggs (Wilson *et al.* 1998). This finding suggested that contamination rates might vary between different parts of the UK.

**1.3** The retail survey in England in 1995/96 showed that there had been no significant change in *Salmonella* contamination of UK-produced eggs since the previous survey in 1991. Following these findings the ACMSF set up the second *Salmonella* in eggs working group in 1998. The working group were aware that vaccination of chickens against *S. Enteritidis* commenced in the mid 1990s and that the number of laboratory confirmed cases of human salmonellosis in the UK, particularly those due to *S. Enteritidis* PT4, showed a steady decrease from 1998 onwards (ACMSF 2001). This trend has continued, and laboratory reports of *S. Enteritidis* PT4 are now at their lowest level since the late 1980s (Cogan and Humphrey 2003).

**1.4** The second ACMSF report on *Salmonella* in eggs published in 2001 concluded that the reduction in the number of laboratory-confirmed cases of salmonellosis was probably mainly due to the impact of vaccinating laying hens against *S. Enteritidis* under the British Egg Industry Council (BEIC) Lion code scheme. However, it should be noted that some producers who are not under the BEIC Lion code scheme also vaccinate against *Salmonella*. It is estimated

that at least 80% of all laying hens in the UK are vaccinated against *S. Enteritidis*. In addition, it should also be recognised that the earlier introduction of controls on laying flocks and improved biosecurity could also have made a contribution to the reduction in human salmonellosis. In their second report, the ACMSF recommended that surveillance should be undertaken to assess whether the overall level of *Salmonella* prevalence of eggs has reduced since the 1995/96 survey and therefore the present survey was undertaken to address this recommendation. The primary objective of the survey was therefore to determine the level of *Salmonella* prevalence of UK-produced shell eggs on retail sale. The design of the survey was based on detecting a 50% reduction in *Salmonella* contamination compared to the 1995/96 survey. The possibility of comparing *Salmonella* prevalence between countries and between production types (e.g. caged, free range, barn, organic) was also considered when planning the survey design although it is important to emphasise that this was not the main objective.

## 2. SURVEY DESIGN

**2.1** The survey was designed to take into account the number of egg samples that needed to be tested to achieve the objective of the survey. This in turn depended on the degree to which the contamination rate had changed since the last survey in 1995/96. To detect a 50% reduction in *Salmonella* contamination from the prevalence in 1995/96 Agency statisticians calculated that 2500 boxes of 6 eggs should be tested. To detect a 25% reduction in *Salmonella* contamination the figure would have risen to 20000 boxes of six eggs. Since the degree of change was unknown, it was decided that a 50% reduction was a realistic target.

**2.2** The sampling plan was initially based on 2500 boxes of 6 eggs. These would be sampled according to the proportion of the market share for each production type as follows according to Defra data for 2000 (Defra Egg Statistics notices):

- Caged 69%
- Free Range (excluding organic) 23%
- Barn 6%
- Organic 2%

**2.3** However, in order to gain enough data to investigate differences between production types, it was necessary to over-sample the minority production types (barn, free range and organic). The core sample for this survey was therefore 3600 boxes, made up of 1800 boxes of caged eggs and 600 samples each of free range, barn and organic eggs.

**2.4** The UK core sample of 3600 was distributed amongst the countries, according to a weighed percentage based on population size multiplied by egg consumption (National Food Survey 2000) and these were as follows:

- England 2966 boxes (82.4%)
- Scotland 339 boxes (9.4%)
- Wales 180 boxes (5.0%)
- Northern Ireland 115 boxes (3.2%)

**2.5** The Food Standards Agency in Scotland and Northern Ireland required additional sampling in order to provide data that could be examined in terms of the different countries. It was decided that the sample size should be 778 in both Scotland and Northern Ireland. These figures were based on 600 boxes to provide an effective sample size for the individual country, plus the additional 178 samples to even out the distribution of the minority production types

across these countries. This gave a total of 4702 boxes, with the following additional samples required in each of the countries.

- Scotland 439 boxes
- Northern Ireland 663 boxes

**2.6** The original sample plan is shown in Annex E. This was calculated based on the total market share amongst the retailers taking into account, as far as possible, sales of the individual production types at these outlets and the availability of stores across the UK.

**2.7** The lead contractor, Direct Laboratories Services, Wolverhampton, ensured that the appropriate number of samples were collected in accordance with the sampling plan and that samples were distributed as uniformly as possible throughout the survey period. To facilitate sampling, the lead contractor designed a regional sampling plan and informed the Agency of any deviations from this, for example, due to a retailer not being available in a particular area or a retailer not having a particular production type. The sampling plan was amended accordingly as the project progressed whilst maintaining a geographical and retailer split as close as possible to the original sampling plan. The final sampling plan showing the changes is shown in Annex F. As far as possible, retail outlets were not sampled more than once and from most outlets no more than four samples were collected from any one store. Where possible, a variety of price ranges were sampled and samplers did not collect samples from retail outlets when they were unsure of the production types.

**2.8** Retailers received payment for the eggs at the time of sampling and contractors were responsible for organising these payments. After purchase of the samples, retailers or a representative of the owner of the premises were given a Food Standards Agency Food Survey leaflet to inform them that samples had been taken from their premises in order to carry out a survey. For larger retail chains, a letter was sent to the relevant contact at the head office with a list of their premises from which samples would be obtained for the survey. The Agency provided retailers and brand owners with survey results obtained from their retail outlets prior to publication of the report.

### **3. SAMPLING**

#### **3.1 Sample Collection and Transportation**

**3.1.1** Experienced samplers were employed to collect samples for the survey. National Milk Records (NMR) were responsible for purchasing and transporting samples to Direct Laboratories in Wolverhampton for England and Wales. Some of the egg samples were taken from local retail outlets by laboratory staff at Direct Laboratories. Charis Food Research and Queen's University Belfast (QUB) were responsible for purchasing, transporting and testing samples in Scotland and Northern Ireland respectively. Retail sampling of eggs was carried out from 2<sup>nd</sup> March 2003 to 8<sup>th</sup> July 2003.

**3.1.2** Samplers collected eggs of UK origin and these were identified on each box of eggs by an initial "9" in the packing station number. For samples that were bought in trays i.e. those from market stalls and farm shops, samplers made enquiries from the retailers to ensure that the eggs collected were UK produced.

**3.1.3** During collection of the eggs, samplers ensured that cross-contamination was minimised by taking precautions at all stages to ensure that the equipment used during sampling, transportation and storage of the eggs was not contaminated with *Salmonella*. For example, by transporting 6 egg samples in separate bags within a box, prevented contamination during transportation.

**3.1.4** Samples consisted of intact eggs with no evidence of damage or contamination. Samplers examined the eggs without touching the contents in the box and rejected any boxes of damaged eggs. Each box was placed in a separate sterile sampling bag to avoid the risk of cross-contamination during transport and storage. Each sampler was expected to sample boxes of 6 eggs. Larger boxes were only sampled when boxes of 6 were not available and the extra eggs discarded at the laboratory.

**3.1.5** The contractor prepared a regional sampling plan and the appropriate number of different retailers for each region was then selected. The plan included details of the retailers, details of egg production system, sampler details, weeks in which sampling visits were to be made, number of boxes of eggs to be collected on each visit. The sampling plan was designed to ensure that the volumes of eggs to be tested were similar for each week.

**3.1.6** Samplers visited each retailer during the agreed week to collect appropriate samples and all relevant information was added to the sampling form (Annex D). They aimed to collect samples in a pseudo-random fashion from the shelves and not necessarily at the front of the display. There was no requirement to select specific sizes of eggs, as there was no available scientific

evidence of any association between egg size and the prevalence of *Salmonella*. Information on whether the eggs were displayed in a temperature control environment was also recorded.

**3.1.7** At some retail outlets (e.g. market stalls or farm shops) it was not possible to buy pre-packaged eggs and retailers packed boxes of eggs from larger trays on demand. These were examined as above. Samplers noted that it was not a legal obligation for retailers to state the production types when selling eggs loose in this way, and therefore endeavoured to find out the production types of the eggs from the retailers when buying these eggs.

**3.1.8** The contractors put in place a contingency plan to ensure that enough samples were collected (e.g. to allow for any eggs broken in transit) by purchasing extra boxes of eggs from the retailers when required.

**3.1.9** Each box of 6 eggs was labelled with a peel-off adhesive label attached to the sampling form and packed into a clean unused polythene bag. The bags were each sealed and packed into insulated boxes along with foam cushioning. Ice packs were added where necessary to ensure the temperatures of the eggs remained between 5° and 20°C during transport to the laboratory. Samples were kept dry and out of direct sunlight. A temperature data logger was placed with some of the samples to monitor compliance with these requirements. Samples were normally delivered to the testing laboratory within 24 hours of sampling.

**3.1.10** Samples from England and Wales were sent to Direct Laboratories in Wolverhampton. Samples from Scotland were sent to the Charis Food Research in Auchincruive and samples from Northern Ireland were sent to the Queen's University in Belfast for *Salmonella* testing.

## **3.2 Sample Information**

**3.2.1** All relevant information available from the sample was entered onto a sampling form (Annex D). The information on the form included details of the retailer, sampler details, date and time of purchase, size of eggs, pack size, packing station number, lion code, purchase date, production types, best before date, price and brand name. Other information included, the temperature of the egg storage area of the laboratory, a reference sampling number, a laboratory number for each sample and the date and time the sample was received in the laboratory. The form also included a section for the laboratory *Salmonella* test results. The contractor entered all data onto a purpose-designed database then converted the data onto an Excel 97 spreadsheet. The updated Excel spreadsheets were sent to the Agency on a fortnightly basis.

**3.2.2** Each sample was given an unique identification code that included letters (e.g. EF2000 England, Free range), in order to clearly link it to a

particular sample and production type. The reference number was retained throughout testing and also when *Salmonella* isolates were sent for typing to the Health Protection Agency (HPA) Laboratory of Enteric Pathogens, Colindale.

## 4. METHODOLOGY

### 4.1 Receipt of Samples

**4.1.1** On receipt at the laboratory, the samples were taken to a dedicated egg storeroom or specimen reception and the temperature data logger information was recorded. The temperature of the storeroom or specimen reception was monitored regularly to ensure that it did not exceed 20°C.

**4.1.2** Most eggs reached the laboratory within 24 hours of sampling. In exceptional situations (e.g. journeys from some Scottish Islands) this period was extended to within 48 hours. The temperature of the eggs were held between 5° - 20°C and the laboratory testing procedure was expected to begin within 24 hours of arrival at the laboratory. All eggs were expected to be tested within their best before dates at the time of analysis.

**4.1.3** Laboratories completed their section of the sample form and any other information that was not entered by the sampler. Additional information on the form included date and time sample was received at the laboratory, appearance of the eggs, Defra packing station number (starts with a "9" for UK produced eggs), egg size, Lion code or any other markings, organic reference number and address of packer/producer where available etc.

**4.1.4** A high-resolution digital photograph was taken of the egg packaging and stored on CD-ROM under the appropriate sample number for samples collected in England, Wales and Scotland. This ensured that the labelling details could be checked at a later date. Where this was not possible the contractor was expected to retain all packaging (or a photocopy/photograph of it). In Northern Ireland, the egg packaging was scanned. All photographs and scanned images of the egg samples were sent to the Agency after the data had been logged

### 4.2 Egg Examination

**4.2.1** On receipt at the laboratory, eggs were examined visually before testing. This was to confirm the absence of cracks and any eggs with marks (e.g. faecal material, feathers, dust, mud, blood and the contents of other eggs) other than natural markings or printed marks were considered dirty. If any egg from a box of 6 was considered dirty or cracked, then the entire box was discarded and one of the contingency samples was used in its place. In the 1995/96 egg survey there were no statistically significant differences in *Salmonella* prevalence between clean and 'dirty/soiled' eggs. Laboratory staff were expected to check that eggs collected were within their best before date on the day of testing.

**4.2.2** Laboratory staff ensured that there was no cross-contamination between boxes and from the surrounding environment. Disposable gloves were worn and changed between each box of six eggs. Thorough cleaning and disinfection of equipment and work surfaces was undertaken regularly. Environmental testing of the laboratory for *Salmonella* contamination prior to egg testing was also carried out regularly throughout the survey.

**4.2.3** All three contractors used a dedicated laboratory for testing of the egg samples. The laboratory control strain used was *Salmonella* Poona, a serotype rarely associated with eggs

### **4.3 Microbiological Analysis**

**4.3.1** The standard operating procedures used for *Salmonella* testing by the contractor is set out in Annex C. Preliminary work was carried out prior to the survey to determine the most appropriate method for testing the shell and contents of eggs for *Salmonella*. Details of this work and comments by the ACMSF are presented in Annex H and I respectively. The Agency considered the ACMSF's comments and also consulted with the relevant industry stakeholders on the methodology. The conclusion was that there are no satisfactory techniques that could effectively differentiate between *Salmonella* contamination on the shell and that in the contents and the methods used in the validation study (Annex H) had the potential to give false positives or negative results. In view of this, the Agency decided that the method used should be the same as in the previous UK egg surveys (de Louvois 1993; ACMSF 2001). This would give an indication as to the relative proportion of contamination on the shell or in the contents and was considered to offer the best chance of picking up all *Salmonella* contaminated eggs. There was also the advantage that the method would allow a direct comparison to be made with the 1995/96 survey in which shell and contents were tested separately. It is recognised that contamination of the shell is likely to occur more commonly than contamination of the contents because there are more opportunities for faecal and environmental contamination to occur. Confirmed isolates of *Salmonella* were sent to the Health Protection Agency (HPA) *Salmonella* Reference Unit within the Laboratory of Enteric Pathogens (LEP), Colindale, for serotyping, phage typing, antimicrobial resistance screening and archiving.

**4.3.2** Direct Laboratories in Wolverhampton, Charis Food Research in Auchincruive, Ayr and the Food Microbiology Unit of Queen's University, Belfast (QUB) are all laboratories accredited by the United Kingdom Accreditation Service (UKAS) to undertake microbiological tests of food and animal feeds for *Salmonella*. In addition to UKAS accreditation, all three laboratories participate in External Proficiency Testing Schemes and routinely test external QA samples. Direct Laboratories participates in the Food Examination Performance Assessment Scheme (FEPAS<sup>®</sup>) for microbiological examination for which test materials are real food samples such as beef, chicken, fish etc. or freeze dried cultures. Charis participates in the Health

Protection Agency (formerly PHLS) Food EQA Scheme which offers a regular series of freeze dried simulated food samples of known but undisclosed content for proficiency testing to challenge everyday laboratory procedures. QUB participates in the Quality in Microbiology Scheme run by QM who are an independent UKAS accredited provider of proficiency testing schemes for microbiology and chemistry for food, water and environmental industries. All of these schemes involved proficiency samples for *Salmonella* in food

#### **4.4 Survey specific External Quality Assurance (EQA) samples**

**4.4.1** Central Science Laboratory (CSL) York were contracted to prepare and label 3 batches of 10 samples (total of 30 samples) of pasteurised liquid whole egg with varying cell concentrations. Each sample was given a code generated by the Agency. The pasteurised liquid whole eggs were inoculated with low levels of either *Salmonella* Poona or *Escherichia coli* in preparation for detection and enumeration tests by participating laboratories. Once the pasteurised liquid whole egg samples had been inoculated, they were stored at 4°C until dispatch (with each batch containing an ice pack to maintain a cool temperature).

**4.4.2** The samples were sent to the three contractor laboratories blinded and a separate “blind” set was sent to a different microbiology laboratory at CSL, York. Only the Food Standards Agency (FSA) and the CSL preparation laboratory knew how many samples were positives with their sample codes.

**4.4.3** In each laboratory every technician involved in the testing of eggs examined at least two of the blinded EQA samples. Prior notice was not given to the laboratories as to when the samples were due to arrive and how many samples would be tested. The FSA also visited each of the laboratories during the course of the survey to assess how the work was being carried out.

**4.4.4** Batches of EQA samples were inoculated and dispatched on Monday 3rd March 2003, Monday 14th April 2003 and Monday 19th May 2003. The samples were dispatched using the Royal Mail special delivery service and arrived at each laboratory before midday on a Tuesday.

**4.4.5** To ensure quality of the samples, CSL analysed 10 coded samples for each dispatch. The first batch had the same coding as those sent to the participating laboratory and the second and third batches were coded separately. CSL recovered the target organism from all the ‘positive’ samples and none from the ‘negative’ samples.

#### **4.5 Zoonoses Order 1989**

**4.5.1** Under the Zoonoses Order 1989, laboratories, which isolate *Salmonella* from foodstuffs, must provide Defra (Scottish Executive Environment and Rural Affairs Department (SEERAD) in Scotland and Department of Agriculture and

Rural Development in Northern Ireland (DARDNI)) with details of the subtype found together with the name of the retailer where the eggs were purchased.

## **4.6 Statistical Methods**

**4.6.1** The final sample distorted the real market share for country and production type (see Chapter 2). For all statistical analyses the individual samples were weighted according to the market shares. Weighted data analyses adjust the raw survey data to represent the population from which the sample is drawn.

**4.6.2** Agency statisticians estimated the prevalence of *Salmonella* contamination of boxes of 6 eggs for potential factors of interest. The prevalence was estimated in percentages and frequencies in the form 1 per  $n$  boxes of 6 eggs, with  $n$  rounded to the nearest 10 with their respective upper and lower 95% confidence intervals.

**4.6.3** *Salmonella* prevalence between different factor of interest were compared using Fisher's exact test (2-tailed) and, where appropriate, p-values were corrected for multiple comparisons using a Bonferroni correction.

**4.6.4** The prevalence of *Salmonella* per box was considered to be more reliable than estimating prevalence at the individual egg level because the survey was designed to test boxes of 6 eggs. To estimate the contamination rate of individual eggs we modelled the box prevalence as a binomial variate with a complementary log-log link function, with offset log (batch size) (Farrington, 1992). This method is likely to under-estimate the correct prevalence at the individual egg level. The results per egg should be seen as a "best case" scenario.

## **4.7 Data Handling and Reporting**

**4.7.1** At fortnightly intervals the lead contractor submitted to the Agency a spreadsheet containing details of the samples collected to date. A summary report on the survey was received from the contractor on a monthly basis. Data on serotyping and phage typing and antimicrobial resistance of *Salmonella* isolates were received from the LEP and the results incorporated into the spreadsheet by the contractor.

**4.7.2** In order to ensure a high level of accuracy in data entry, the Agency ensured that the contractor provided a specific standard operating procedure (SOP) and distributed this to all the participating laboratories. Staff inputting data were trained and experienced with the database package. All data were entered into an Excel spreadsheet and checked by the laboratory manager at

Direct Laboratories Services. Data entry was controlled through a purpose-designed database with in-built checks to minimise data entry errors.

**4.7.3** The lead contractor was responsible for collating all the results and a draft final spreadsheet of the survey results was sent to the Agency on 31 July 2003. Data provided to the Agency was then cross-checked against the photographs and sampling forms to ensure that they were accurate. Further random spot checks of 120 of 4753 (2.5%) sample datasets were also performed by the Agency. Each dataset was cross-checked for accuracy after discussion with Agency statisticians.

## 5. SURVEY RESULTS

### 5.1 Distribution of samples in the survey

**5.1.1** 4753 boxes of six or more eggs were sampled in the survey and the shell and contents of these eggs were tested for *Salmonella*. This figure excludes four packs of 4 eggs (the microbiological analysis required 6 eggs from the same box) which were purchased and tested but were later removed from the survey, leaving a final figure of 4753 samples. The final sample figure was higher than expected due to over-sampling in the different production types and retailers, however in cases other than the boxes of four eggs, it was relevant to include the data in the survey. In terms of production types 50.0% were caged, 16.9% were free-range, 16.6% were organic eggs and 16.5% of the eggs were barn.

**5.1.2** 63% of the egg samples were purchased in England, 17% in Scotland, 16% in Northern Ireland and 4% in Wales. Table 1 below summarises the number of samples of each production type collected from different parts of the UK.

**Table 1: Summary of total number of egg samples of each production type that were included in the survey.**

Country	Production Types				Total
	Barn	Caged	Free Range	Organic	
England	497 (16.7%)	1496 (50.2%)	494 (16.6%)	494 (16.6%)	<b>2981 (62.7%)</b>
Northern Ireland	130 (16.6%)	389 (49.7%)	133 (17.0%)	130 (16.6%)	<b>782 (16.5%)</b>
Scotland	130 (16.0%)	401 (49.4%)	148 (18.2%)	132 (16.3%)	<b>811 (17.1%)</b>
Wales	28 (15.6%)	90 (50.3%)	30 (16.8%)	31 (17.3%)	<b>179 (3.8%)</b>
<b>Total</b>	<b>785 (16.5%)</b>	<b>2376 (50.0%)</b>	<b>805 (16.9%)</b>	<b>787 (16.6%)</b>	<b>4753 (100.0%)</b>

### 5.2 Packing stations

**5.2.1** The eggs were packed at various packing stations and the packing station registration number was recorded from the egg boxes. Businesses covered by the EU regulations on marketing standards for hen eggs are required to register premises where eggs are packed and must place their unique registration number on the label of each box of eggs.

**5.2.2** These numbers can therefore be used to trace eggs back to the packing

station. The first number in the packing station code indicates the country of origin and the number “9” represents the UK. The second number indicates the UK region and the final number refers to the actual packing station. This number can also be suffixed with a letter to further define it as a substation within the same location or group. If an egg had not been packed at a packing station because it was sold direct to the final consumer by the producer, at a market for example, then it might not have a code on the label.

**5.2.3.** The retail eggs sampled in the survey came from 259 identified packing stations, 149 (57.5%) in England, 56 (21.6%) in Scotland, 35 (13.5%) in Wales and 19 (7.3%) in Northern Ireland. In addition, 4.1% of the samples (194) did not have packing station numbers.

**5.2.4** There were five UK packing stations in the survey that also pack non-UK eggs (packing stations 9/0/402, 9/8/135, 9/8/971, 9/5/066 and 9/4/463). Their details were checked with Defra and it was confirmed that they had eggs in the past, but were no longer doing so. A total of 26 samples originating from these packing stations were purchased in the survey, of which 22 were not Lion coded. The Egg Marketing Inspectorate (EMI) within Defra confirmed that all these eggs were produced in the UK and they were therefore included in the survey.

### **5.3 Pack size and egg size**

**5.3.1** The 4753 egg samples comprised 4396 boxes of 6 eggs, and 357 egg samples (8%) from boxes larger than 6 eggs. Out of the 4396 boxes of 6 there were 42 samples (1%) where the eggs were sold from trays.

**5.3.2** Samplers collected packs of various sizes of eggs, ranging from small to extra large eggs. The percentages of eggs of different sizes sampled during the survey were 41.2% medium, 30.8% large, 20.8% mixed sized, 4.2% extra large, 2.7% small, 0.2% unknown and <0.1% very large. Where the egg sizes were unknown this was because it was not stated at the point of sale or on the box.

### **5.4 Retailers**

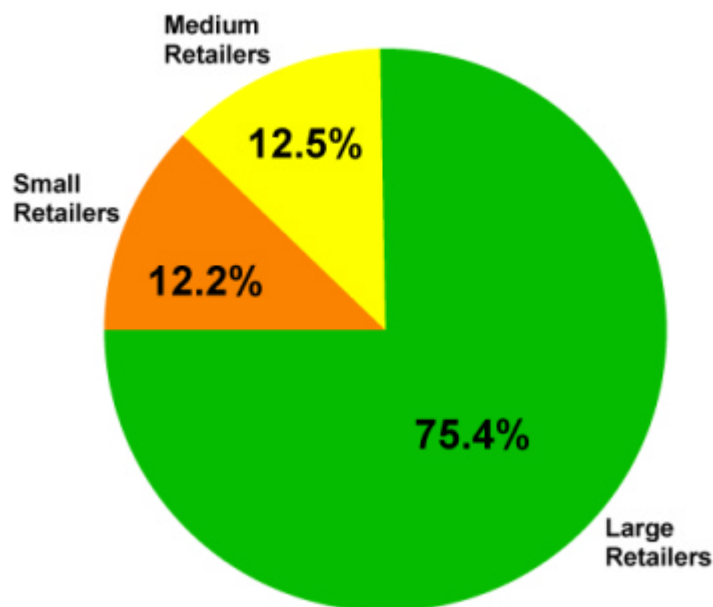
**5.4.1** The retailer categories were classified according to market share by the Agency and can be described as:

- **Large** - Supermarkets/Large retailers
- **Medium** – Independent/Local shops, small chains and multiples
- **Small** – Farm shops, market stalls, butchers, milkmen, garages, mobile grocer vans, greengrocers.

**5.4.2** Of 4753 boxes of eggs included in this survey, 75.4% were from large retailers, 12.5% from medium retailers and 12.2% from small retailers.

**5.4.3** Figure 1, compares the sampling plan based on the market share of egg sales within the UK in 2002 with the actual number of samples taken during the course of the survey. The market share was defined through the UK egg data supplied by Taylor Nelson and Sofres (TNS) (see Annex A Tables A1-A12 for raw figures).

**Figure 1: The proportion of eggs sampled from different retailer categories based on the market share of egg sales**



**5.4.4** The distribution of samples taken in the survey differed slightly from the original plan, with 5% fewer samples taken at large retailers and 5% more at smaller retailers than expected. This change in the sampling plan was due to barn, free range and organic eggs not being available at some of these outlets. There was also a regional effect where retailers in some regions tended to be smaller outlets, which led to more samples being collected at these retail shops. From the data collected, 28 retailers sold their own brand of eggs.

**5.4.5** In terms of best before date, 96.6% of all the eggs purchased carried a best before date either on the egg or the packaging. For production types, 90.6% of barn, 98.5% of caged, 96.4% of free range and 97.1% of organic eggs carried a best before date.

## 5.5 Production Systems

**5.5.1** The samples in the survey comprised 50.0% caged production 16.9% free range 16.6% organic and 16.5% barn production. However, due to the over production of some egg types, cascading down of eggs may occur i.e. organic/free range eggs may be cascaded down to barn. Due to the practice of cascading, the production type at retail level is an assumption made from the labelling on the egg box. The frequency of cascading is unknown, although it is not considered to be a common practice. Where the egg boxes did not have the production types stated on the boxes, the retailer provided information on the production type to the sampler. There were 8 samples that fell into this category.

**5.5.2** The sampling plan was designed on the basis of statistics provided by TNS (see Annex A Tables 1-12) for raw figures of the breakdown by country of sampling. A comparison between the projected sampling plan and actual samples taken by production type is shown in Table 2 below.

**Table 2: The number of egg samples from different production systems relative to the sampling plan\***

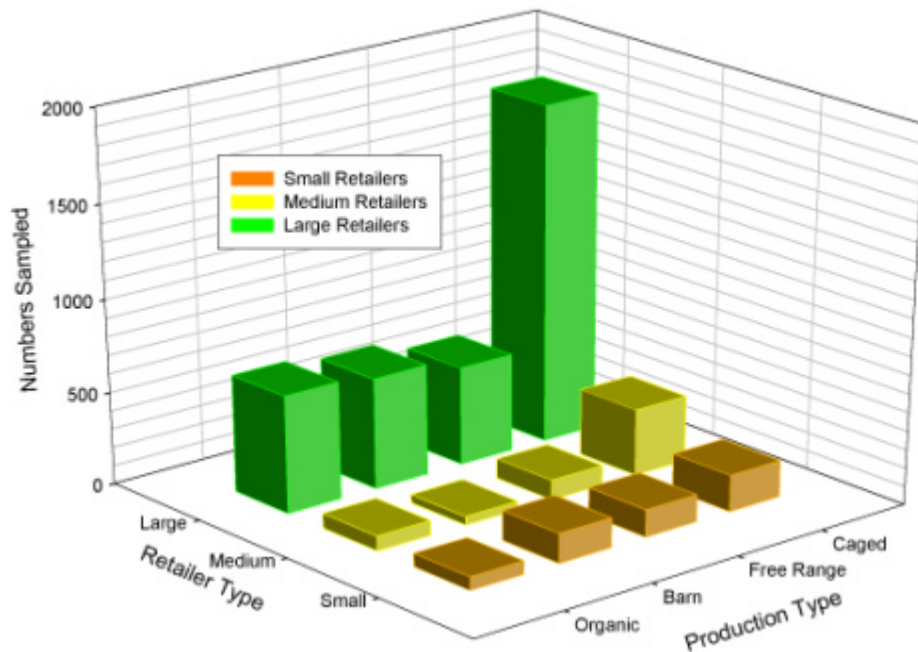
<b>Production System</b>	<b>Sampling plan</b>	<b>Survey sample</b>
Barn	784 (16.6%)	785 (16.5%)
Caged	2350 (50.0%)	2376 (50.0%)
Free range	784 (16.6%)	805 (16.9%)
Organic	784 (16.6%)	787 (16.6%)

\* A more detailed breakdown is provided in Annexes E and F

**5.5.3** Figure 2 shows the number of samples purchased by size of retailer and production types. Caged eggs were the most sampled production type, reflecting the market share of this category. However, difficulties were encountered in obtaining sufficient samples of other production types especially in Scotland and Northern Ireland. Figure 2 highlights the fact that barn eggs were hard to obtain from medium and smaller (except in farm shops) retail outlets and had to be obtained from larger retailers and farm shops.

In Scotland and Northern Ireland there were difficulties in obtaining organic eggs from large retail outlets, as they did not stock them. Also, during sampling, it was observed that organic eggs were less common in Northern Ireland, especially amongst the smaller retail outlets e.g. butchers.

**Figure 2: Number of samples in the survey according to egg production types and retail outlet in the UK**



## 5.6 Egg Assurance schemes

**5.6.1** 4030 (84.8%) of the egg samples had the Lion Code mark on the egg boxes, which indicated that the eggs were laid by hens vaccinated against *S. Enteritidis* or an *S. Enteritidis* / *S. Typhimurium* combined vaccine as part of the BEIC's Lion Code scheme. The proportion of non-Lion Code eggs that were from vaccinated layers is not known. Six (0.13%) of the egg samples were labelled as having been produced under the "Laid in Britain" Quality Assurance scheme of the United Kingdom Egg Producers (UKEP).

## 5.7 Storage conditions

**5.7.1** For 95% of the samples the eggs were kept at ambient temperatures at retail outlets with only 5% sold chilled. Further breakdown of the data shows that in the case of chilled eggs these comprised 2.4% caged, 1.0% free range, 0.9% organic and 0.7% barn. Samples sold at ambient temperatures comprised 50.1% caged, 16.7% barn, 15.9% free range and 15.7% organic eggs.

## 5.8 *Salmonella* positives

**5.8.1** A total of 14 egg samples were reported as positive for *Salmonella* contamination and in all cases these were from the shell. Five of the positive samples were of *Salmonella* Dublin, which although common in cattle is very rarely seen in poultry, whether layers or broilers. *Salmonella* Dublin was not isolated in the previous UK egg surveys in 1991 (de Louvois, 1993), 1995/96 (ACMSF 2001), 1996/97 (Wilson *et al.* 1998 or in recent testing of UK and non-UK eggs from catering establishments by the HPA (PHLS 2003). The *Salmonella* Dublin isolations were unusual and unexpected and a thorough investigation was carried out to try to establish whether the presence of *S.* Dublin was likely to be a true finding. This is detailed in Annex Q.

**5.8.2** *Salmonella* Dublin belongs to the same serogroup as *S.* Enteritidis. Although rarely found in poultry, there remains a theoretical possibility that such a contamination event might occur on one or possibly two occasions. However, this would not explain the 5 positive samples originating from a number of different retail outlets, packing stations, producers and geographically scattered flocks. Moreover, detailed follow-up testing of surviving or replacement flocks and their environment failed to find any evidence of *S.* Dublin contamination on the farms that produced the implicated eggs. Molecular typing suggested that the five isolates were indistinguishable and comparable to recent isolates seen in cattle (Annex P and Q). On this basis, it was concluded that, whilst a definitive explanation could not be provided for the presence of *S.* Dublin on eggs, it was most likely to have occurred as a result of cross-contamination during handling and/or testing. The Agency therefore considers that there is sufficient doubt about the validity of the *S.* Dublin results to exclude them from the main analysis of the survey. However, a statistical analysis of the survey data including the five *S.* Dublin positive samples is provided in Annex R for completeness. It is important to emphasise that the main conclusions to be drawn from the survey remain the same with or without the *S.* Dublin positive samples.

**5.8.3** Of the nine remaining positive samples, seven were purchased in England and two in Wales. There were no positive samples from Scotland and Northern Ireland. The nine positives were from six packing stations and six retail outlets. Table 3 summarises the positive results in terms of country of sampling, packing station, *Salmonella* serotype, retailer, brand name and price of the eggs. *Salmonella* positive samples were all from the caged production type and from the large and medium retailer category.

**Table 3: Information on the *Salmonella* contaminated egg samples from retail outlets.**

Date sampled	Country Where Sampled	Retailer size*	Price per 6 eggs	Packing Station number	Production type	<i>Salmonella</i> Serotype
04/03/03	England	Large	£0.52	9/1/998H	Caged	Enteritidis
12/03/03	England	Large	£0.85	9/1/993A	Caged	Enteritidis
17/03/03	England	Medium	£0.89	9/1/998N	Caged	Livingstone
07/04/03	England	Large	£0.44	9/1/998M	Caged	Enteritidis
07/04/03	England	Large	£0.44	9/1/998M	Caged	Enteritidis
10/04/03	England	Medium	£0.85	9/1/998H	Caged	Enteritidis
24/04/03	England	Large	£0.49	9/1/993A	Caged	Enteritidis
04/06/03	Wales	Medium	£0.67	9/8/135	Caged	Enteritidis
20/06/03	Wales	Medium	£0.69	9/4/463	Caged	Infantis

\*See section 5.4.1 for classification of retailer size

**5.8.4** *Salmonella* positives in England were all Lion Coded eggs, whilst those in Wales did not have the Lion Code mark on the eggs or egg boxes. All positive samples were from eggs displayed at ambient temperature and from pack sizes of 6 eggs, except for one sample, from packing station 9/8/135, which was from a pack size of 10.

## 5.9 *Salmonella* serotypes and phage types.

**5.9.1** The 9 *Salmonella* isolates comprised 3 different serotypes (Table 4). Seven of the 9 (78%) isolates were *S. Enteritidis* and comprised of 3 phage type (PT) 4 isolates, one PT6, one PT12, one possible PT23, and one untypeable isolate.

**5.9.2** Table 3, also shows the packing station numbers relating to the *Salmonella* positive samples. The nine positive samples came from 6 packing stations supplying 30% (998/4753) of the eggs sampled in the survey. The *S. Enteritidis* PT4 isolates came from 2 packing stations supplying 8.8% (417/4753) of the eggs sampled in the survey. Three packing stations each packed 2 egg samples that tested positive for *Salmonella* Enteritidis.

**Table 4: Details of the *Salmonella* subtypes found in the survey\***

<i>Salmonella</i>	Phage types	Number of
S. Enteritidis	All phage types	7
S. Enteritidis	4	3
S. Enteritidis	6	1
S. Enteritidis	12	1
S. Enteritidis	Untypeable	1
S. Enteritidis	Possibly 23	1
S. Infantis	-	1
S. Livingstone	-	1

\* England and Wales data

<sup>1</sup> Five isolates of *S. Dublin* were excluded (see section 5.8.1)

## 5.10 Antimicrobial susceptibility testing

**5.10.1** All *Salmonella* isolates were tested for sensitivity to a panel of 10 antimicrobial drugs using a breakpoint method (Threlfall *et al.* 1999). The breakpoint concentrations for the antimicrobial drugs are shown in Annex K.

All isolates including those of *S. Dublin* were fully sensitive to the agents at the concentrations used. In addition, the three isolates of *S. Enteritidis* PT4 were screened for rifampicin resistance since this is a marker for the live *S. Enteritidis* vaccine strain used in the layer sector. None of the isolates showed evidence of rifampicin resistance. The HPA LEP considered that the isolates did not conform to the resistance pattern of known vaccine strains.

## 5.11 External Quality Assurance Results

**5.11.1** Direct Laboratory Services, Charis Food Research, Queen's University of Belfast and Central Science Laboratory analysed 10 coded samples for each dispatch. All participants recovered the target organism from all the 'positive' samples and none from the 'negative' samples.

**5.11.2** No false positives or false negatives were recorded from the quality assessment of participating laboratories.

## 6. STATISTICAL ANALYSIS

### 6.1 Statistical analysis of survey results

**6.1.1** Statistical analysis of the contamination rate of *Salmonella* in a box of 6 eggs showed that the overall prevalence of *Salmonella* in a box of six eggs was 0.34% for the UK as a whole, i.e. around 1 box in every 290 boxes.

### 6.2 Prevalence of *Salmonella* per box of 6 eggs

**6.2.1** Table 5 shows the prevalence of *Salmonella* in a box of 6 eggs and the 95% confidence intervals (CI) (the lower and upper values) for the UK, England, Wales, Scotland and Northern Ireland. The lower and upper values represent the boundaries of the 95% confidence interval assuming that the observed prevalence followed a binomial distribution.

**6.2.2** There were no statistically significant differences in *Salmonella* prevalence between England, Wales, Scotland and Northern Ireland (all  $p > 0.10$ ). The UK figure of 0.34% (95% CI: 0.17% - 0.62%) is equivalent to 1 in 290 boxes (95% CI: 1 in 590 boxes to 1 in 160 boxes).

**6.2.3.** It should be noted that Wales had the highest *Salmonella* prevalence (1.53%) and the smallest sample size of all the countries (179 samples) The consequences of this are that:

- The confidence interval for *Salmonella* prevalence for eggs sampled in Wales is wider than the other countries.
- To identify statistically significant difference between Wales and the other countries, the *Salmonella* prevalence for eggs sampled would have to be at least 2%.

**Table 5: The prevalence of *Salmonella* per box of 6 eggs in the UK and in England, Wales, Scotland and Northern Ireland.** Figures shown include 95% confidence intervals.

Country (sample size)	Lower 95% CI	Prevalence	Upper 95% CI
<b>UK (4753)</b>	<b>0.17%</b>	<b>0.34%</b>	<b>0.62%</b>
England (2981)	0.13%	0.32%	0.65%
N. Ireland (782)	0.00%	0.00%	0.61%
Scotland (811)	0.00%	0.00%	0.58%
Wales (179)	0.21%	1.53%	5.25%

### 6.3 Prevalence of *Salmonella* Enteritidis and *S. Enteritidis* PT4

6.3.1 The prevalence of *Salmonella*, *S. Enteritidis* and *S. Enteritidis* PT4 per box is shown in Table 6. The *S. Enteritidis* and *S. Enteritidis* PT4 prevalence figures are equivalent to 1 in every 370 and 910 boxes respectively.

**Table 6: The prevalence of *Salmonella* Enteritidis and *Salmonella* Enteritidis PT4 per box of 6 eggs.** Figures shown include 95% confidence intervals.

	Lower 95% CI	Prevalence	Upper 95% CI
<b><i>Salmonella</i></b>	<b>0.17%</b>	<b>0.34%</b>	<b>0.62%</b>
S. Enteritidis	0.12%	0.27%	0.52%
S. Enteritidis PT4	0.03%	0.11%	0.31%

### 6.4 *Salmonella* prevalence and egg production types

6.4.1 Table 7 shows the prevalence of *Salmonella* contamination per box of 6 eggs for the four production types. Although *Salmonella* contamination was only found on eggs from caged production, no statistically significant differences in *Salmonella* contamination of eggs was found between the four production types ( $p > 0.20$ ). The *Salmonella* prevalence of caged eggs would have had to be at least 1% to show statistically significant differences from other production types.

**Table 7: The prevalence of *Salmonella* per box of 6 eggs from caged, free range, barn and organic production systems.** Figures shown include 95% confidence intervals.

Production type (sample size)	Lower 95% CI	Prevalence	Upper 95% CI
Barn (785)	0.00%	0.00%	0.56%
Caged (2376)	0.24%	0.50%	0.92%
Free Range (805)	0.00%	0.00%	0.56%
Organic (787)	0.00%	0.00%	0.56%

## 6.5 Prevalence of *Salmonella* per egg

**6.5.1** The UK figure for individual egg prevalence was 0.06% for *Salmonella* (95% CI: 0.03% - 0.10%). The estimated prevalence of *Salmonella* per egg was based on the assumption that the rate of cross-contamination between eggs within the box was equivalent to the contamination rate between boxes. Given the low prevalence of *Salmonella* within boxes, we can assume that the source of contamination within a box of 6 eggs is from one egg only. In performing this calculation the assumption is that, although eggs are packed in a quasi-random order, the presence of more than one contaminated egg in a box is unlikely to occur frequently when the incidence of contamination is as low as in this study. In reality this may not be the case and the overall figure may be somewhat higher than indicated. This assumption might underestimate the true *Salmonella* prevalence at single egg level.

## 6.6 *Salmonella* prevalence for other factors of interest

**6.6.1** The following factors of interest were considered to evaluate whether there were differences in presence/absence of *Salmonella* on eggs:

- Lion Code (Yes, No)
- Size of retail outlet (Large, Medium, Small) (as categorised in 5.4.1)
- Storage temperature (Ambient, Chilled);
- Price per egg;
- Date of purchase;
- Remaining shelf-life (best before date - date of purchase).

**6.6.2** Since the survey was not designed to compare *Salmonella* prevalence among these factors, all results should be considered with care. To enable the calculation of *Salmonella* prevalence and 95% confidence intervals in each factor one important assumption was made:

- The egg market shares for individual countries and production types (see Chapter 2) were considered to be constant in the different categories within the factors of interest. For example, in the storage temperature factor, chilled eggs were assumed to have the same distribution across the UK and across the different product types as the ambient eggs. In some cases this may be an unlikely assumption, so reported prevalence and confidence intervals should be interpreted with care.

**6.6.3** Table 8 shows the prevalence of *Salmonella* in a box of 6 eggs and the 95% confidence intervals for Lion Code/Non-Lion Code eggs, ambient/chilled eggs and large/medium/small retailers. There were no statistically significant

differences in *Salmonella* prevalence between Lion Code/Non-Lion Code eggs ( $p > 0.30$ ) and between ambient/chilled eggs ( $p > 0.50$ ). However, medium category retailers had a statistically significant higher prevalence than large retail outlets ( $p = 0.028$ ). No other retail size comparison was statistically significant (all  $p > 0.10$ ).

**Table 8: The effect of Lion Code, storage temperature and retailer category on the prevalence of *Salmonella* per box of 6 eggs.** Figures shown include 95% confidence intervals.

Factors of Interest (sample size)	Lower 95% CI	Prevalence	Upper 95% CI
<b>Lion Coded (4753)</b>			
Yes (4030)	0.12%	0.29%	0.59%
No (723)	0.09%	0.63%	2.13%
<b>Storage Temperature (4753)</b>			
Ambient (4515)	0.17%	0.35%	0.63%
Chilled (238)	0.00%	0.00%	5.87%
<b>Retail Category (4753)</b>			
Large (3582)	0.08%	0.23%	0.52%
Medium (593)	0.46%	1.58%	3.90%
Small (578)	0.00%	0.00%	1.11%

**6.6.4** To calculate the prevalence and 95% confidence intervals for the continuous variables (price per box, date of purchase, remaining shelf-life), the data were divided into broad meaningful categories.

- Price per box was divided into two categories: boxes that cost less than £1 and boxes that cost more than or equal to £1.
- Date of purchase was divided into 3 categories of 30 days each and a fourth category of 39 days. Each 30 day period represented approximately a month of the sampling period, except for the fourth category.
- Remaining shelf-life was divided into 2 categories: 14 days or less and more than 14 days. This was based on dividing the 28 days shelf life of eggs in two.

**6.6.5** Table 9 shows the prevalence of *Salmonella* in a box of 6 eggs and the 95% confidence intervals for price per box, date of purchase and remaining shelf-life. There were no statistically significant differences in *Salmonella* prevalence in the price per box ( $p > 0.90$ ), in the date of purchase (all  $p > 0.10$ ) and in the remaining shelf-life ( $p > 0.10$ ).

**Table 9: The effect of price, date of purchase and remaining shelf-life on the prevalence of *Salmonella* per box of 6 eggs.** Figures shown include 95% confidence intervals.

Factors of Interest (sample size)	Lower 95% CI	Prevalence	Upper 95% CI
<b>Price per box (4744)</b>			
Less than £1 (3670)	0.17%	0.35%	0.65%
More or equal than £1(1074)	0.00%	0.00%	3.32%
<b>Date of purchase (4753)</b>			
First 30 days (1773)	0.11%	0.36%	0.90%
Second 30 days (1278)	0.03%	0.44%	1.86%
Third 30 days (1172)	0.00%	0.00%	0.58%
Fourth 39 days (530)*	0.00%	0.25%	2.04%
<b>Remaining shelf-life (4591)</b>			
Less or equal than 14 days (1745)	0.21%	0.55%	1.15%
More than 14 days (2846)	0.05%	0.21%	0.56%

\*Includes the last nine days of the survey.

## 6.7 Comparison with the 1995/96 survey

**6.7.1** The findings from the samples taken in England in this survey were compared to those in the previous egg survey in England in 1995/96. Statistical analysis showed that the prevalence of *Salmonella* contamination of eggs in the 2003 survey was statistically significantly lower than the prevalence in 1995/96 ( $p < 0.001$ ). Table 10 compares the *Salmonella* prevalence for the two surveys at the box level using England data from the 2003 survey. A statistical comparison with the findings of an earlier survey in England and Wales 1991 (de Louvois 1993) has not been undertaken as the earlier survey only sampled eggs from high street retail outlets.

**Table 10: The prevalence of *Salmonella* per box of eggs in England in 2003 compared to the previous survey in 1995/96.** Figures shown include lower and upper 95% confidence intervals.

<b>Box Level</b>			
	Lower 95% CI	Prevalence	Upper 95% CI
2003 England	1/770 boxes	1/310 boxes	1/150 boxes
2003 England	0.13%	0.32%	0.65%
1995/96	1/120 boxes	1/100 boxes	1/90 boxes
1995/96	0.83%	0.99%	1.17%

## 7. DISCUSSION

### 7.1 Overall results of the survey

**7.1.1** The results of this survey showed a statistically significant reduction in *Salmonella* contamination of UK produced eggs in England compared to the previous egg survey in 1995/96. With only 9 isolates throughout the survey, the overall prevalence of *Salmonella* in a box of 6 eggs was 0.34% for the UK as a whole. This would suggest that the package of measures, including vaccination, put into place by the UK egg industry, since the early 1990s has had a significant impact on *Salmonella* in the laying sector.

**7.1.2** Because the survey was carried out at retail, it is not possible to establish the exact proportion of eggs tested in the survey that were vaccinated. 84.8% of the samples tested were Lion Coded (vaccination of laying hens against *S. Enteritidis* is compulsory in this scheme). However, vaccination status cannot be ascertained for those eggs which were not Lion Coded and therefore the results will tend to underestimate the effect of vaccination because some of the non-Lion Coded eggs will have been from vaccinated flocks. This could in part account for the lack of any statistically significant difference in the rate of *Salmonella* isolations between Lion Code and non-Lion Code eggs although the smaller number of non-Lion Code eggs sampled could also be a factor. In terms of non-Lion-Coded eggs, very few of these (0.13%) were labelled as being produced under the "Laid in Britain Quality Assurance" scheme. The scheme is aimed specifically at independent egg producers, and is run by the UK Egg Producers (UKEP) Ltd. It is reported to use a competitive exclusion and HACCP-based approach for the control of *Salmonella*.

**7.1.3** There was also no statistically significant difference in *Salmonella* contamination between eggs from different production types although *Salmonella* positive samples were only found in samples from caged production, which represented 50% of the egg samples in the survey. There were no statistically significant differences in *Salmonella* prevalence in the price per box ( $p > 0.90$ ), in the date of purchase (all  $p > 0.10$ ) and in the remaining shelf-life ( $p > 0.10$ ). There were no statistically significant differences in *Salmonella* prevalence between Lion Code/ non-Lion Code eggs ( $p > 0.30$ ) and between ambient /chilled eggs ( $p > 0.50$ ). However, medium category retailers had a statistically significant higher prevalence than large retail outlets ( $p > 0.028$ ).

**7.1.4** Temperature at retail level, appearance of the eggs prior to testing and time remaining before the best before date appeared to have no significant impact on whether eggs were positive for *Salmonella* or not.

**7.1.5.** Although the findings in paragraphs 7.1.2 - 7.1.4 are real statistical effects it is important to emphasise that the underlying sample contains a small number of positive egg samples and in some comparisons there were large differences in the numbers of samples examined.

**7.1.6** It should be noted that it is not unusual for *Salmonella* to be present in the environment and therefore not surprising that a few isolates were found. The small number of positive samples points towards some contamination from the production environment rather than any systemic contamination from infected flocks. Davies and Breslin (2003) have shown that *Salmonella* can persist on layer farms even after the introduction of vaccination for *S. Enteritidis*.

## **7.2 Characteristics of the serotypes isolated**

**7.2.1** Of the nine *Salmonella* isolates on which this report focuses, the serotypes found were those known to have been associated with eggs before. These were mainly *Salmonella* Enteritidis but there were also single isolates of *Salmonella* Livingstone and *Salmonella* Infantis. These findings were not unexpected as both *S. Livingstone* and *S. Infantis* were isolated in the previous UK and non-UK egg surveys (de Louvois 1993; ACMSF 2001)

**7.2.2** Seven of the isolates were *Salmonella* Enteritidis, and three of these were phage type 4 (PT4). It is this serotype that was the major focus of concern in the early nineties as it made up the largest proportion of *Salmonella* isolates from human cases, including those linked to eggs (ACMSF 2001). Vaccination was specifically introduced to provide a control measure for this phage type in line with other biosecurity measures, such as environmental monitoring, disinfection, etc, which would have had an impact on all salmonellas. The vaccines are also expected to show activity against other phage types of *Salmonella* Enteritidis and other Group D serotypes of *Salmonella*. Other *Salmonella* Enteritidis phage types isolated in the survey were PT6, PT12, a probable PT23 and an untypeable isolate. PT6 was isolated in the 1995/96 survey and has been found recently in human cases in an outbreak in 2003 linked to eggs.

**7.2.3** Five samples were excluded from the overall results of the survey as their serotyping results and further testing confirmed *Salmonella* Dublin. This is a highly unusual and unexpected finding, as this serovar has never been isolated in previous surveys of UK and non-UK eggs and is normally associated with cattle and to a lesser extent sheep. In addition, the farms from which the samples were taken were all dedicated layer farms, which

subsequently all tested negative for *Salmonella* Dublin. It is not possible to rule out the occurrence of *S. Dublin* on eggs on theoretical grounds, however, follow-up investigations have cast doubt on the validity of the finding. The data have therefore been excluded from the overall results and the results and further investigations reported in Annexes P and Q. This could have implications for the findings from the statistical analysis, the analysis was re-run with the *S. Dublin* isolates included. Apart from differences in the prevalence of contamination the main statistical findings remain unchanged.

**7.2.4** Antimicrobial resistance profiles indicated that all of the isolates were sensitive to the antibiotics tested. This is not surprising, as previous egg surveys have shown that antimicrobial resistance is uncommon in isolates of *S. Enteritidis* from UK-produced eggs (de Louvois 1993).

### **7.3 Distribution of the isolates**

**7.3.1** Of the 9 positive samples, 7 were purchased in England and 2 in Wales. All but two of these isolates originated from large supermarket chains, which accounted for the vast majority of eggs sold at retail sale (see Table 3). The findings do not suggest any recognisable geographical pattern across the UK. Statistical analysis indicated that there was a significantly higher prevalence of *Salmonella* positives in “medium-sized” retailers. Although this finding is based on a small number of positives it would repay further investigation.

**7.3.2** The packing stations from which the positive eggs originated were all based in England, as are the large majority of all packing stations (see 5.2.3). There does not appear to be an obvious link between the *Salmonella* isolates based around the 6 packing stations implicated due to the different time-points when *Salmonella* was isolated from the samples. Three of the packing stations had packed two of the positive egg samples each, which in two cases were destined for the same retailer. The other three packing stations had packed one of the positive egg samples each.

### **7.4 Nature of the contamination of eggs that tested positive**

**7.4.1** Of the eggs that tested positive, all of the isolates were found on the outside of the shell and it is encouraging that there were no positive isolates in the contents of the eggs. Due to limitations with the methodology, an absolute distinction cannot be drawn between contamination of the egg shell and contents and reporting all positives together without distinguishing shell from contents also facilitates comparison with previous surveys. However, separate shell and content testing was carried out at the request of the egg industry, following consultation on the protocol.

**7.4.2** Despite commissioning work to evaluate the best method of testing both shell and contents and taking the advice of the ACMSF on the basis of these results (Annex H and I), it is not possible to be certain that either part would not contaminate the other. Further information is given in Annex J. Nevertheless, these results do give us a very good indication that *Salmonella*, if present, is more likely to be due to environmental contamination of the shells. Although, shell contamination may not initially appear to be an issue, as consumers do not eat shells, there is still a risk of cross-contamination to other foods and surfaces in the kitchen and into the contents when the egg is broken. FSA advice to consumers is that eggs are raw products that should be handled with care. Consumer perception may be that handling of shells does not represent a risk to food safety. The Agency has commissioned research to look at this issue although it seems likely that there is a potential risk of cross-contamination from the surface of the egg to the contents, ready to eat foods, work surfaces and containers if standard hygiene advice is not followed. *Salmonella* Enteritidis is also well adapted to survive in egg contents, and has the potential to survive and grow if stored incorrectly or for too long.

## **7.5 Traceback of positive samples**

**7.5.1** All of the egg samples that tested positive were traced back to their original source. The trace back was conducted, after the results had been thoroughly cross-checked, analysed and verified, via the retailers where the eggs were purchased (in most cases this was also the brand owner). The retailers were asked to provide details of packing stations, farms if known and the flocks. The results of this traceback provided no common factors linking the small number of positive samples in the survey.

## **7.6 Non-UK eggs**

**7.6.1** There was little evidence of non-UK eggs being sold at retail outlets and these were excluded from the survey. The numbers of packing stations which the Egg Marketing Inspectorate were aware had packed non-UK eggs at some stage in the past were provided to the Agency. In the cases where samples were identified as originating from these packing stations and they were not Lion-Coded (i.e. it was not absolutely certain they were from the UK). This only occurred on 21 occasions confirming that retail is a very uncommon source for these eggs. Two of the eggs that may have been of non-UK origin tested negative but it was not possible to draw any conclusions from these results as the origin is not guaranteed and the numbers are so few.

**7.6.2** In Autumn 2002, whilst this survey was being planned, there were a number of outbreaks of salmonellosis in the UK, which were mainly linked to eggs used at catering outlets (PHLS 2003). On a number of occasions the source of the eggs was traced to Spain. The Health Protection Agency followed up these outbreaks by carrying out testing on eggs taken from the

outlets linked to the outbreaks. In 2003 the HPA set up a co-ordinated study with Local Authorities Co-ordinators of Regulatory Services (LACORS) to sample and test eggs from catering establishments. Whilst this provides valuable information on the potential for contamination of non-UK eggs it is not the purpose of the HPA study to establish the source of the eggs or to compare eggs from different countries with the UK.

**7.6.3** The Agency intends to undertake a survey of non-UK eggs incorporating a sampling plan that will allow enough eggs to be taken from individual countries in order to compare them with this survey of UK produced eggs. From January 2004, under new EU legislation, all eggs must be stamped indicating the country in which they were produced. In addition, there will be a requirement to label egg boxes with a code indicating the country in which they were packed. This will assist in identifying non-UK eggs at whatever point in the chain they are sampled from. The Agency hopes to begin this non-UK egg survey in 2004.

## **7.7 Comparison with previous egg surveys**

**7.7.1** Table 11 provides a comparison between the three major surveys of *Salmonella* contamination of UK-produced eggs on retail sale. For comparative purposes the data in Table 11 has been adjusted to contain numbers sampled for England in the 2003 survey. The data shows the substantial reduction in *Salmonella* contamination of eggs between the 1995/96 and 2003 surveys and, in particular, the reduction in *S. Enteritidis* PT4.

**Table 11: Comparison between the *Salmonella* subtypes isolated in the 1991, 1995/96 and 2003\* retail surveys of UK-produced eggs.**

	England & Wales		England		UK
	1991 <sup>+</sup>	2003	1995/6	2003	2003
Total boxes tested	7,054	3,160	13,970	2,981	4,753
<b>No. samples positive for: <i>Salmonella</i> Enteritidis</b>					
PT4	33 (0.47%)	3 (0.13%)	82 (0.58%)	3 (0.14%)	3 (0.11%)
Other PTs	15 (0.21%)	4 (0.17%)	37 (0.26%)	3 (0.14%)	4 (0.15%)
<b>Total</b>	<b>47 (0.6%)<sup>a</sup></b>	<b>7 (0.30%)</b>	<b>115 (0.82%)<sup>b</sup></b>	<b>6 (0.28%)</b>	<b>7 (0.27%)</b>
<b><i>Salmonella</i> Typhimurium</b>					
DT104	1 (0.01%)	0 (0.00%)	5 (0.04%)	0 (0.00%)	0 (0.00%)
<b>Total</b>	<b>6 (0.09%)</b>	<b>0 (0.00%)</b>	<b>6 (0.04%)</b>	<b>0 (0.00%)</b>	<b>0 (0.00%)</b>
<b><i>Salmonella</i> Infantis</b>					
<b>Total</b>	<b>1 (0.01%)</b>	<b>1 (0.04%)</b>	<b>1 (0.01%)</b>	<b>0 (0.00%)</b>	<b>1 (0.04%)</b>
<b><i>Salmonella</i> Livingstone</b>					
<b>Total</b>	<b>8 (0.11%)</b>	<b>1 (0.04%)</b>	<b>4 (0.03%)</b>	<b>1 (0.05%)</b>	<b>1 (0.04%)</b>
<b>Other <i>Salmonella</i> Types</b>					
<b>Total</b>	<b>3 (0.04%)</b>	<b>0 (0.00%)<sup>c</sup></b>	<b>12 (0.09%)<sup>d</sup></b>	<b>0 (0.00%)<sup>c</sup></b>	<b>0 (0.00%)<sup>c</sup></b>
<b>Total <i>Salmonella</i> detected</b>	<b>65 (0.92%)</b>	<b>9 (0.39%)<sup>c</sup></b>	<b>138 (0.99%)</b>	<b>7 (0.32%)<sup>c</sup></b>	<b>9 (0.34%)<sup>c</sup></b>
<b>Total <i>Salmonella</i> isolates</b>	<b>66 (0.94%)<sup>a</sup></b>	<b>9 (0.39%)<sup>c</sup></b>	<b>144 (1.07%)<sup>e</sup></b>	<b>7 (0.32%)<sup>c</sup></b>	<b>9 (0.34%)<sup>c</sup></b>

<sup>+</sup> High street retailers only

<sup>a</sup> One sample had two different *Salmonella* Enteritidis phage types.

<sup>b</sup> Four samples had two different *Salmonella* strains isolated from shell and contents.

<sup>c</sup> Figures do not include *Salmonella* Dublin isolates which have been excluded from the dataset (see 5.9.1)

<sup>d</sup> Two samples had two different *Salmonella* strains from shell and contents

<sup>e</sup> Six samples had two separate *Salmonella* strains.

## **7.8 Limitations of the sample plan**

**7.8.1** The core sample number was 2500 as this was the minimum number required to observe accurately whether there had been a 50% reduction in *Salmonella* contamination since the previous survey in 1995/96. The survey met its aims as the study was able to show that there had been a greater than 50% reduction from the previous survey. The study was designed to take into account the market share of eggs on an UK-wide basis. Whilst every attempt was made to follow the suggested number of samples, the UK egg market is a fast moving one, and there were occasions where changes to sampling were required. However, whilst certain retailers may have been slightly over- or under-sampled, this is not considered to have had a material effect on the results.

**7.8.2** The map produced showing where sampling took place (see Annex G) clearly indicates that the whole UK has been covered on a population representative basis, with most samples taken from the main conurbations. At no time in the survey did the number of samples taken represent a handling problem for any of the 3 laboratories contracted.

## **7.9 Cross-contamination**

**7.9.1** The Agency originally wished to carry out this survey at packing stations in order to improve traceability of the samples and reduce sampling costs. Unfortunately it was not possible to gain packers' and producers' co-operation to carry out this survey under the terms on which the Agency operates. One of the potential criticisms in this case is that the further away from the packing station samples are taken, the higher the risk that any *Salmonella* found is due to cross-contamination. However, the added advantage of taking samples at retail is that a more realistic picture can be drawn of the eggs entering consumers' kitchens. It should also be noted that the likelihood of consumers carrying *Salmonella* on their hands and these being transferred to eggs when they are handled in the shop is extremely low. Nevertheless, the contractors tried to minimise any such risk by not taking samples from the front of the shelves.

## **7.10 External Quality Assurance**

**7.10.1** As an additional measure the Agency implemented an external quality assurance (EQA) programme. A separate contractor was commissioned to develop and produce the samples for this scheme. On three occasions during the survey ten samples were sent to each of the three laboratories taking part in the survey and blind tested by a laboratory at the contractor who developed the EQA. Some of the samples sent to the laboratories contained very low levels of *Salmonella* Poona (an isolate very rarely found on eggs, which can be distinguished should there be any possibility of cross-contamination).

Further details of the EQA scheme used can be found at Annex L. All three of the laboratories performed to a high standard in these EQA trials and all results were correct.

**7.10.2** It should also be noted that the laboratories all had their own Quality Assurance systems in place and full measures were taken to avoid cross-contamination occurring at all stages of the survey as outlined for Direct Laboratories in Annexes B, C and O.

## **7.11 Impact of the survey findings on FSA advice**

**7.11.1** The FSA's current advice to consumers is that cooking eggs properly will kill any bacteria. This means cooking eggs until both the white and yolk are solid and cooking dishes that contain egg until they are piping hot all the way through (<http://www.food.gov.uk/safereating/foodadvice/eggs2002advice>). The FSA has also recently issued a leaflet for caterers on the use of eggs ([www.food.gov.uk/multimedia/pdfs/eggleaflet.pdf](http://www.food.gov.uk/multimedia/pdfs/eggleaflet.pdf)). General food handling and cooking advice is also given to consumers. The FSA believes that if the above advice is adhered to the risk to consumers from *Salmonella* in eggs is low but it is never possible to guarantee that any food is risk free. The Agency will consider the results of this survey along with those of other surveys (such as the forthcoming non-UK egg survey) and the results of available research when considering its advice to consumers on egg consumption.

**7.11.2** The FSA intends to review the findings of its research programme on eggs in 2004 and as part of this review will consider the findings of funded research projects in relation to the current advice on the use of eggs and egg products.

## 8. CONCLUSIONS

**8.1** In the last major survey, conducted in 1995/96, the eggs were sampled in England only. On this occasion eggs were sampled from all four countries in the UK. If the findings from the current survey are compared on an England only basis then there has been a 3-fold reduction in the level of *Salmonella* contamination since 1995/96. This is likely to reflect the measures introduced by the UK egg industry to control *Salmonella*.