



**Survey of *Salmonella* Contamination of Non-UK
Produced Shell Eggs on Retail Sale in the North West
of England and London.**

Final Report - Project B18012

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Contents

Glossary	4
List of Tables	5
List of Figures	6
List of Annexes	7
Acknowledgements	8
Executive Summary	9
1.0 Introduction	11
2.0 Methods	15
2.1 Survey design	15
2.2 Sampling	17
2.2.1 Sample collection and transportation	17
2.2.2 Sample information	19
2.2.3 Sample receipt	20
2.2.4 Sample examination and isolation of <i>Salmonella</i> spp.	21
2.2.5 External Quality Assurance (EQA) samples	22
2.2.6 Zoonoses Order 1989	23
2.2.7 Data handling and reporting	23
3.0 Results	25
3.1 Approach to statistical analysis	25
3.2 Collection and examination of non-UK egg samples in the survey	26
3.2.1 Regional distribution of non-UK egg samples in the survey	26
3.3 Prevalence of <i>Salmonella</i> in non-UK eggs at retail sale	27
3.3.1 Prevalence of <i>Salmonella</i> spp., <i>S. Enteritidis</i> , <i>S. Enteritidis</i> PT1 and non-PT1 at 'box' level	28
3.4 Country of origin in relation to presence of <i>Salmonella</i> spp.	29
3.5 <i>Salmonella</i> isolated from both shells and contents – serotypes, phage types and antimicrobial susceptibility	29
3.6 Sampling period in relation to presence of <i>Salmonella</i> spp.	

in non-UK eggs at retail sale	31
3.7 Egg production details in relation to presence of <i>Salmonella</i> spp.	33
3.7.1 Condition of non-UK eggs in relation to production details and retail premises type	35
3.8 Retail premises details in relation to presence of <i>Salmonella</i> spp.	35
3.9 Shelf-life details of non-UK egg samples in relation to presence of <i>Salmonella</i> spp.	36
3.9.1 Storage temperature of eggs at retail premises	37
3.10 Traceability of non-UK eggs sampled at retail premises	38
4.0 Discussion	40
5.0 Conclusions	48
6.0 References	49
Annexes to the report	54

Glossary

ACMSF	Advisory Committee on the Microbiological Safety of Food
BBD	Best before date
BEIC	British Egg Industry Council
BEIS	British Egg Information Service
BGA	Brilliant green agar
BPW	Buffered peptone water
CI	Confidence Intervals
Defra	Department for Environment Food and Rural Affairs
DH	Department of Health
EC	European Commission
EEDD	Environmental and Enteric Diseases Department
EFSA	European Food Safety Authority
EHD	Environmental Health Department
EHO	Environmental Health Officer
EMI	Egg Marketing Inspectorate
EQA	External Quality Assurance
EU	European Union
FEPTU	Food and Environmental Proficiency Testing Unit
FSA	Food Standards Agency
HACCP	Hazard Analysis and Critical Control Points
HPA	Health Protection Agency
LACORS	Local Authority Co-ordinators of Regulatory Affairs
LAs	Local Authorities
LEP	Laboratory of Enteric Pathogens
LIMS	Laboratory information management systems
MKTTn	Muller-Kauffmann Tetrathionate Novobiocin broth
MRA	Microbiological Risk Assessment
PT	Phage Type
QA/QC	Quality assurance / Quality control
RDNC	Reacted but did not conform
RVS	Rappaport-Vassiliadis soya peptone broth
SOP	Standard Operating Procedure
TSO	Trading Standards Officer
UKAS	United Kingdom Accreditation Service
XLD	Xylose lysine desoxycholate agar

List of Tables

- Table 1. Regional distribution of non-UK eggs purchased during the survey
- Table 2. Prevalence of *Salmonella* contamination in non-UK eggs (n=1,744 pools of eggs)
- Table 3. Estimated prevalence of *Salmonella* contamination per box of 6 non-UK eggs on retail sale
- Table 4. Country of origin of non-UK egg samples and *Salmonella* positive pools
- Table 5. *Salmonella* sero- and phage types recovered from non-UK egg shells and contents (n=173)
- Table 6. *Salmonella* isolates recovered from the shell and contents of eggs produced in France, Poland and Spain (n=173)
- Table 7. Number of non-UK egg samples purchased during the sampling period and number of *Salmonella* positive pools
- Table 8. Production details of non-UK egg samples and *Salmonella* positive pools
- Table 9. Retail premises details where non-UK egg samples were purchased and *Salmonella* positive pools
- Table 10. Remaining days until expiration of best before date of non-UK egg samples and *Salmonella* positive pools.
- Table 11. Traceability details of non-UK egg samples and no. of *Salmonella* positive pools

List of Figures

Figure 1. Sampling period in relation to presence of *Salmonella* spp. in non-UK eggs

List of Annexes

- Annex A: Food Standards Agency Survey of *Salmonella* Contamination of Non-UK Produced Shell Eggs on Retail Sale: Protocol
- Annex B: Food Standards Agency Survey of *Salmonella* Contamination of Non-UK Produced Shell Eggs on Retail Sale: Letter provided to retailers during sampling
- Annex C: Food Standards Agency Survey of *Salmonella* Contamination of Non-UK Produced Shell Eggs on Retail Sale: Sampling Form
- Annex D: Definition of a Class A Egg, Egg Stamp Mark Requirements, and Article 33 of Regulation (EC) 2295/2003 – Tolerance for Quality Defects
- Annex E: Egg Production Systems
- Annex F: Breakpoint Concentrations for Antimicrobial Drugs
- Annex G: Non-UK Egg Import Share and Sample Returns
- Annex H: *Salmonella* Contamination of Shell and Contents Samples From Non-UK Eggs (n=1,890)

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Executive Summary

The Food Standards Agency's (FSA's) 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* serotypes and phage types and their antimicrobial susceptibility. The study also looked at whether there were any associations between types of *Salmonella* and the country of origin of eggs.

There is no market share data on non-UK eggs at the retail level in the UK and therefore no basis on which to construct a sampling frame. However, there was some market intelligence and this directed sampling in two geographical locations of the UK where non-UK eggs were thought to be available on retail sale. Through the life of the survey it became apparent that the market intelligence was incomplete and that sampling bias has crept into the survey. From the statistical analysis, the results provide an estimate of boundaries or a measured estimate of prevalence of *Salmonella* contamination in non-UK eggs.

A total of 1,890 boxes of 6 or more eggs were sampled during the course of the survey from targeted retail premises in the north west of England and London. Of these 145 samples (i.e. boxes of 6 eggs) had visible cracks and therefore were unacceptable and not included in the main data analysis. One sample was also rejected due to the lack of information on the shell or box. In total 1,744 samples were deemed acceptable for testing. The shell and contents of eggs were tested separately for the presence of *Salmonella*.

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 eggs originating from Portugal, Republic of Ireland, Belgium and Poland.

The overall finding was that 157 samples were contaminated with *Salmonella* spp. 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, *S. Enteritidis* were 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 the survey. From these, eight different serotypes were obtained, of which most were *S. Enteritidis* (84.9%; 147/173). There were nine different phage types (PT) of *S. Enteritidis*, with PT1 predominating (81.6%; 120/147). *S. Enteritidis* PT4 was not detected. Other serotypes detected included *S. Mbandaka* (14), *S. Unnamed* (6), *S. Rissen* (2), *S. Braenderup* (1), *S. Infantis* (1), *S. Panama* (1) and *S. 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%).

It is not unusual for *Salmonella* to be present in the environment and therefore contributing to the contamination of the egg shell. However, 10 samples which were shell positive were also contents positive which suggests that systemic infection with *Salmonella* in some laying flocks where eggs were sampled.

1. Introduction

Food poisoning is often debilitating and occasionally fatal. It also imposes a significant economic burden on a nation in medical costs and lost working time (Adak *et al.* 2002, FSA 2000, Mead *et al.* 1999). Infection with *Salmonella* Enteritidis remains an important public health problem in Europe and some parts of the world (Fisher 2004, Drociuk *et al.* 2003, Mishu *et al.* 1994). Outbreaks caused by *Salmonella* have been associated with a variety of foods. However, outbreaks caused by *Salmonella* Enteritidis are closely 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). Concerns are such that the European Commission (EC) Scientific Committee on Veterinary Measures relating to Public Health has identified eggs and egg products containing raw eggs as a food group which pose a hazard to public health (EC 2003a).

Raw shell eggs may become contaminated with *Salmonella* spp. in several ways. The outside of the egg may be contaminated by faeces after laying, or be infected with *Salmonella* spp. during laying if the reproductive tract is colonised by the organism. *Salmonella* spp. on the outside of the egg can migrate through the porous shell to the interior, particularly when newly-laid or under humid conditions. Contamination of the egg contents can also arise from infection of the reproductive tissue prior to egg development (de Buck *et al.* 2004, Humphrey 1994). Salmonellae can survive in lightly cooked eggs or raw egg dishes to cause human disease (Humphrey *et al.* 1989, 1990). The UK Chief Medical Officer (DH 1988a, 1988b) and Food Standards Agency (FSA) (FSA 2003) have drawn attention to the risk associated with eating raw and lightly cooked eggs and issued public health advice on the safe handling and use of eggs.

In the UK and western Europe, the predominant phage type (PT) responsible for eggborne *Salmonella* Enteritidis infection has been PT4 (Fisher 2004, Gillespie

et al. 2005). Following large epidemics of *S. Enteritidis* in the late 1980s, mainly due to PT4, a significant decline in human *S. Enteritidis* PT4 infection in England and Wales has occurred since 1997, largely due to industry control programmes such as the vaccination of layer flocks and quality assurance schemes incorporating improved biosecurity (ACMSF 2001, Cogan and Humphrey 2003). However, since 2002, the emergence of egg associated *S. Enteritidis* PTs other than PT4 causing human infection has taken place, with the greatest increases occurring in *S. Enteritidis* PT1 and PT14b (HPA 2004, 2005a). Surveillance of salmonellosis from 1998 to 2003 has also identified upsurges in *S. Enteritidis* non-PT4 in other European countries (Fisher 2004). These major resurgences are thought to be associated with major changes in market supply with the import of eggs from other egg producers in Member States 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).

The demand from retail market is almost covered by UK produced eggs produced and therefore the need to supplement demand with non-UK eggs is low. The retail market accounts for approximately 49% of the total volume of eggs used in the UK and has a market value of £514 million per year (BEIS 2005). Compared to the total UK production and usage, the volume of eggs sourced from outside the UK is small but has increased since 1999 (Defra 2006). Almost all non-UK eggs are sourced from other EU Member States and most are destined for processing or to the catering sector with a small proportion intended for retail sale (ACMSF 2001). The volume of non-UK eggs sourced from outside the UK fluctuates throughout the year and closely reflects supply and demand (Defra 2006).

In Europe, *Salmonella* is controlled in the egg production chain under Regulation (EC) No 1168/2006, which sets targets to reduce *Salmonella* prevalence in poultry and eggs in all member states (EC 2006a). It is also planned under this Regulation that from January 2010, eggs from *Salmonella* infected flocks will be

treated in a manner that guarantees the elimination of *Salmonella*, e.g. the heat treatment of contaminated eggs. Contaminated eggs will therefore be required to be sent for processing into egg products. Traceability of all table eggs (Class A) sold in the EU became mandatory in January 2004, in that shells must be stamped with a distinguishing code (EC 2001). During outbreak investigations this code enables a full product traceability by identifying the type of farming system, the country of origin and the precise production establishment (EC 2001).

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 compared to UK-produced eggs. In 1996/97, a survey of non-UK eggs intended for retail sale found that 2% of samples contained *Salmonella* spp., 1.3% contained *S. Enteritidis* and 0.1% contained *S. 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 *S. 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 appear to have decreased significantly, clearly demonstrating an improved situation (1995/6; 1.0%, 2003; 0.3%) (ACMSF 2001, Elson *et al.* 2005, FSA 2004a).

The FSA has set up a series of raw shell egg surveys, this survey of non-UK eggs at the retail level, and another of eggs at the catering level (FSA 2005a, 2005b, 2004b). The results from these surveys will contribute to providing an indication of where particular contamination problems are occurring and hence where interventions to reduce *Salmonella* contamination might best be focused. Reported here are the results of a survey to provide an estimate of *Salmonella*

contamination of non-UK eggs on retail sale in the north west of England and London.

This survey had three principal aims:

- Estimate the prevalence of *Salmonella* in non-UK raw shell eggs at retail sale in the UK;
- Identify the *Salmonella* sero- and phage-types present in non-UK raw shell eggs on retail sale and determine the susceptibility of the isolates to antimicrobial drugs;
- Establish any association between country of origin of eggs and presence of particular sero / phage types of *Salmonella*.

2. METHODS

2.1 Survey design

The main objective of the survey was to determine the level of *Salmonella* contamination of non-UK produced eggs sold at retail. The number of eggs required to be tested to achieve this objective depended on the estimated level of contamination based on the previous survey (ACMSF 2001). Since the degree to which the rate has changed was unknown, the decision was taken to start by testing 2,500 samples of boxes of 6 or more eggs in the course of a year (1 April 2005 – 31 March 2006), FSA statisticians calculated that such an initial sample size would be needed to achieve the required precision required. This was based on the assumption that the estimated prevalence in the survey would be at 1.5%. A 95% confidence interval on this estimate provided a range from a fraction over 1% to a fraction above 2%; i.e. there is a high confidence that the true prevalence lies within the range (1% to 2%) (Annex A). The results were assessed every three months to ensure that enough eggs were being sampled to achieve the best estimated prevalence possible.

Seasonal effects were not investigated; therefore it was acceptable to cover retail premises region by region rather than continually sampling throughout the UK. Information surrounding the supply of non-UK eggs to the market place was vague but intelligence reports seemed to suggest that non-UK eggs could be found at small retail premises in the north west of England and in areas in and around London. In and around these two regions there are several large egg importers which are known to supply eggs of non-UK origin to the retail market. The survey therefore was confined to local authorities and laboratories that are situated in and around London and north west England to increase the likelihood of sampling non-UK eggs from a range of retail premises. The project lead contractor, the Environmental and Enteric Diseases Department (EEDD), HPA Centre for Infections administered the project and together with the HPA London Food, Water & Environmental Microbiology and Chester Food and Environmental

Microbiology Services Laboratories co-ordinated sampling in London and the north west of England.

Any retail premises could be included in the study provided they had non-UK produced raw shell eggs available for sampling. It was generally known that non-UK eggs were not readily available from large multiple retailing chains and these retailers were not targeted in the survey. Identification of retail premises that sold non-UK eggs required a scoping exercise to determine where these eggs were on retail sale. Such scoping exercises were carried out at the start of the survey and at quarterly intervals over the 16 month sampling period, including other regions aside from the north west and London.

Following the sustained upsurge in *S. Enteritidis* non-PT4 infections in England and Wales during 2002-2004, a targeted national risk communication strategy was launched by the Health Protection and Food Standards Agencies in October 2004 to raise awareness among egg importers, caterers and the general public (HPA 2005a). This followed a local HPA initiative in north west England in July and August 2004, where a particularly high number of outbreaks of *S. Enteritidis* PT14b infection had occurred (HPA 2005a). This led to egg importers, particularly in north west England, to stop or reduce the sourcing of non-UK eggs, which also resulted in a lower number of non-UK egg samples being collected during the first quarter of the survey than planned. By agreement with the FSA, sampling within the survey was therefore extended by four months (1 April 2005 – 31 July 2006) with a revised target number of between 1,000 and 2,000 samples of eggs. FSA statisticians assessed the interim results and suggested that the number of samples be revised to 1,000 boxes of 6 eggs to provide a measurement of *Salmonella* levels. The figure would not necessarily provide the true prevalence of *Salmonella* in non-UK eggs but could be used as an indicator of what may be occurring in the non-UK egg population and of any pronounced problems.

Retail premises could only be revisited after a period of at least four weeks to ensure different consignments of eggs were sampled. The number of egg samples/boxes collected per premises related to the amount of eggs on sale. Multiple samples (up to 4) were only collected at retail premises where large numbers of boxes were on display, such as in supermarkets, whereas only one to two samples/boxes were collected from smaller shops.

Retailers received payment for the eggs at the time of sampling. After purchase of the samples, retailers or a representative of the owner of the premises were given a FSA food survey sampling letter to inform them that samples had been taken from their premises in order to carry out a survey (Annex B). At the end of the survey larger retail chains were sent a letter by the FSA to the relevant contact at the head office with a list of their premises from which samples had been obtained. The FSA provided retailers and brand owners with survey results obtained from their retail premises prior to publication of the report.

2.2 Sampling

2.2.1 Sample collection and transportation

Samples were collected by experienced staff from 21 Environmental Health Departments (EHDs) in north west England and London (Barnet, Bexley, Brent, Camden, Congleton, Enfield, Hackney, Harrow, Hillingdon, Hounslow, Lambeth, Liverpool, Macclesfield, Merton, Sefton, St Helens, Tower Hamlets, Vale Royal, Wandsworth, Warrington, Wirral), and by an additional Environmental Health Officer (EHO) specifically recruited to support the London region, between 1 April 2005 and 31 July 2006 using a standardised sampling protocol, sampling form, and sampling plan (Annexes A and C). The testing laboratory organised the sampling to cover regions, months in which sampling visits were to be made, and number of boxes of eggs to be collected each month. The approach was to ensure that the volumes of eggs to be tested were similar for each month.

Non-UK eggs were identified by the stamp marking on the egg shells, e.g. 1AT12345. The distinguishing number includes an indication of origin (e.g. AT, Austria), method of production (e.g. 1, free range), and the farm where the eggs were produced (e.g. 12345, farm ID) (Annex D). Where the farm has multiple hen houses the farm ID numbers can be appended with a letter (e.g. A, B, C and so on). Annex E provides information on egg production systems, i.e. cage, barn, free range and organic. Where eggs were found without the egg stamp, these were informally reported to the Egg Marketing Inspectorate (EMI) for further investigation or action.

All raw shell table eggs sold at retail level must be Class A quality and therefore fit for human consumption. The definition of Class A eggs is provided in Annex D. Where eggs sampled were found to be in breach of the Class A marketing standards, including eggs on sale with expired best before dates, these findings were reported to the FSA on an informal basis who in turn contacted the EMI for further investigations. At the same time, these findings were also informally reported to Local Authority Trading Standards Officers (TSOs) by the participating local authorities for further investigation or action.

Samples were collected at random 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.

Samples consisted of intact eggs with no evidence of gross damage. Samplers visually examined the eggs avoiding contact with the contents in the box and rejected any boxes of grossly damaged eggs. Each box was placed in a separate sterile sampling bag to avoid the risk of cross-contamination during transport and storage. For the purpose of this survey, a sample was equivalent to a box of six eggs but larger boxes (e.g. 10's or 12's) were sampled if different

brands were available or that was all that was on sale. The extra eggs from the larger boxes were discarded at the laboratory. At some retail premises, such as market stalls, it was not possible to buy pre-packaged eggs and these retailers packed the boxes of eggs from larger trays at the point of sale. A contingency plan was put in to place 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. The extra boxes were not tested and not included in this survey where the primary sample arrived undamaged.

During collection of the egg samples, cross-contamination was minimised by taking precautions at all stages to ensure that the equipment used during sampling, transport and storage was not contaminated with *Salmonella*. Samples were kept dry and out of direct sunlight, transported at approximately 20°C and delivered to the testing laboratories within 24 hours of sampling. Samples were collected and transported to the laboratory in accordance with the Food Law Code of Practice and Practice Guidance (FSA 2006), advice provided in LACORS guidance on microbiological food sampling (LACORS 2006), and the FSA Sampling Protocol (Annex A).

Samples of non-UK raw shell eggs were transported to two Official Food Control Laboratories; samples in London were dispatched to the HPA London Food, Water & Environmental Microbiology Services Laboratory, and those in north west England to the Chester HPA Collaborating Food and Environmental Microbiology Services North West.

2.2.2 Sample information

All relevant information available from the sample was recorded on a sampling form (Annex C). The information included details of the retailer, sampler details, date and time of purchase, storage temperature of eggs, size of eggs, pack size, packing station number, country of origin, purchase date, production types, best before date, price, vaccination scheme, and brand name. Other information

included, the visual condition of the eggs, the stamp marking on the egg shell, a reference sampling number, a laboratory reference number for each sample and the date and time the sample was received in the laboratory. The form also included a section for the testing laboratory to enter the *Salmonella* test results. The laboratories entered data in to Laboratory Information Systems (LIMS), converted the data onto an Excel[®] spreadsheet and forwarded on a monthly basis to the HPA EEDD, Centre for Infections, for central data collation and analysis.

The laboratory reference sample number acted as a unique identification code in order to clearly link it to a particular sample. The reference number was retained throughout testing and also when *Salmonella* isolates were sent for typing to the HPA Laboratory of Enteric Pathogens (LEP), Centre for Infections.

2.2.3 Sample receipt

Egg samples reached the laboratory within 24 hours of sampling. On receipt at the laboratory, the samples were taken to a dedicated specimen reception and the temperature data logger information was recorded. The temperature of the eggs were held at approximately 20°C and the laboratory testing procedure began within 24 hours of arrival at the laboratory.

Most eggs at the time of both collection and examination were within their best before dates. Eggs with expired best before dates at the time of collection were also examined, to reflect the quality eggs available to the consumer, these represented a small proportion of the overall sample number.

Laboratories completed their section of the sample form, which included the date sample was received at the laboratory, stamp marking on the egg shell, and appearance of the eggs, i.e. whether they were clean, dirty, contaminated with faeces or visibly cracked (MAFF 1996b) (Annex D).

Digital photographs of high resolution were also taken of the egg packaging and stamp mark on the eggshell. Photographs were stored under the appropriate laboratory reference sample number for samples. This ensured that the labelling and stamp mark details could be checked at a later date. All photographs of the egg samples were stored on CD-ROM and sent to the FSA on a monthly basis for further validation and cross checking by the FSA project officer.

2.2.4 Sample examination and isolation of *Salmonella* spp.

Laboratory staff ensured that there was no cross-contamination between boxes and from the surrounding environment. Disposable gloves were worn and changed between each sample 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.

In addition, raw meat or poultry samples or QA/QC samples containing strains of *Salmonella* spp. were not tested at the same time or before any raw shell eggs were tested. The eggs were cracked in a designated area of the laboratory away from routine food testing. Guidance on 'avoiding cross contamination risks in the food laboratory' was provided in a HPA guidance note (HPA 2005b). The laboratory control strain used was *Salmonella* Nottingham, a serotype rarely associated with foods including eggs.

Samples of six eggs were aseptically cracked against the rim of a sterile screw topped container; the shells and egg contents were divided and pooled in separate containers. The presence of *Salmonella* was tested in accordance with BS EN ISO 6579: 2002 (BSI 2002). An equal volume of buffered peptone water (BPW) was added to both contents and shell samples and the solutions mixed and then incubated at 37°C for 18 ± 2 hours. The incubated BPW broths were then sub-cultured into selective enrichment broths; 0.1 ml to 10 ml of Rappaport-Vassiliadis Soya Peptone Broth (RVS) and 1 ml to 10 ml of Muller-Kauffmann

Tetrathionate Novobiocin broth (MKTTn) and incubated at 41.5°C and 37°C, respectively for 24 ± 3 hours. After incubation, the broths were sub-cultured on to Xylose Lysine Desoxycholate agar (XLD) and modified Brilliant Green Agar (BGA) plates. Inoculated selective agars were incubated at 37°C for 24 ± 3 hours and then examined for typical and atypical or suspect colonies of *Salmonella*. Serological and biochemical confirmation were carried out on typical or suspect colonies of *Salmonella* from each agar plate. Isolates of *Salmonella* spp. were referred to HPA, LEP for confirmation, serotyping and phage typing for *S. Enteritidis* (Ward *et al.* 1987), antimicrobial susceptibility testing (Frost 1994, Annex F), and archiving. Detailed methodology was further provided in the HPA standing operating procedure for the detection of *Salmonella* species (HPA 2005c).

The method used was similar to that of previous surveys of *Salmonella* contamination of eggs in which shell and contents were tested separately (FSA 2004a). It should be noted that there is no method of sample preparation currently in existence that reliably allows the separation of egg shell and egg contents and thus distinguishes shell from content contamination. However, as part of a pan-London study during a public health investigation on eggs, the method has been shown to be robust when using aseptic techniques. This has been shown where shells were tested positive some samples were also contents negative. In previous study contents have been found positive with shells testing negative. (personal communication - HPA). The results will therefore be reported as a rate per group of six eggs as in previous surveys. The separate data for shell and contents contamination are included in the report for interest.

2.2.5 External Quality Assurance (EQA) samples

Both the HPA London Food, Water & Environmental Microbiology Laboratory, and the Chester HPA Collaborating Food and Environmental Microbiology Services North West are Official Food Control Laboratories and as such are accredited by the United Kingdom Accreditation Service (UKAS) to undertake

microbiological tests of food for *Salmonella* (http://www.food.gov.uk/enforcement/public_analysts/foodcontrollabs). Both laboratories thus participate in the HPA Food External Quality Assessment (Proficiency Testing) Extended scheme (EQA). Full details on the scope of EQA schemes are available from the HPA website (HPA 2006).

In the extended scheme a minimum of 12 simulated food samples are dispatched per year at two-monthly intervals with two samples per distribution, i.e. two samples are dispatched every two months. The extended scheme offers a regular series of freeze dried simulated food samples of known but undisclosed content for proficiency testing to challenge everyday laboratory procedures. The survey protocol required the testing of EQA samples by a different technician on three occasions throughout the survey, details of which were provided to the FSA at the end of the survey. The laboratories also run internal quality assurance programmes in which all laboratory staff participate. These examine spiked samples that include spikes of *Salmonella* spp. in various food matrices. Guidance on the 'recommended minimum internal quality control in food microbiology testing laboratories' is provided in a HPA guidance note (HPA 2005d).

2.2.6 Zoonoses Order 1989

Under the Zoonoses Order 1989, laboratories which isolate *Salmonella* spp. from foodstuffs, must provide Defra with details of the subtype found together with the name of the retailer where the eggs were purchased (MAFF, 1989). Details of *Salmonella* spp. isolated from non-UK eggs were notified to Defra by HPA EEDD.

2.2.7 Data handling and reporting

In order to ensure a high level of accuracy in data entry, laboratory staff trained and experienced with the Laboratory Information Management Systems (LIMS)

inputted data. All data were then transferred and/or entered into an Excel® spreadsheet and validated by HPA EEDD.

At monthly intervals HPA EEDD submitted a spreadsheet containing details of the samples collected to date, together with a summary and/or bimonthly report on the survey to the FSA. Data on serotyping and phage typing and antimicrobial susceptibility of *Salmonella* isolates were received from the HPA LEP and the results incorporated into the spreadsheet by HPA EEDD. Data provided to the FSA were then cross-checked by Agency staff against the photographs to ensure that they were accurate. Further random spot checks of sample datasets were also performed by the FSA. Each dataset was cross-checked and corrected for accuracy after discussion with the FSA.

3. RESULTS

3.1 Approach to statistical analysis

There is no market share data on non-UK eggs at the retail level in the UK and therefore no basis on which to construct a sampling frame. However, there was some market intelligence data and this directed the sampling to two geographical locations of the UK where non-UK eggs were thought to be available on retail sale. Through the life of the survey it became apparent that the market intelligence was incomplete and that sampling bias had crept into the survey. and the lack of market share data, the results from the statistical analysis provide an estimate of boundaries or a measured estimate of prevalence of *Salmonella* contamination in non-UK eggs.

Although the sampling carried out broadly reflected the availability of the non-UK eggs on retail sale (as thought by market intelligence) it can not be considered representative of the UK market. Market intelligence directed sampling to two geographical areas (to increase the likelihood of sampling non-UK eggs) and specific retailers, reflecting the fact that most non-UK eggs are imported through wholesalers based in the north west of England and London and that these traders supply independent shops i.e. not major supermarkets or food retail chains. Given that the sampling was not carried out randomly as retailers were selected through scoping exercises, the estimated prevalence will be biased in some way. The results from the analysis provide an estimate of the prevalence of *Salmonella* in non-UK eggs based on several assumptions:-

- *The eggs' country of origin proportions are the same in the retail sector as they are for overall imports;*
- *The export country is the country of origin for non-UK eggs;*
- *The proportions of eggs sourced per non-UK country seen in 2005 also hold true for 2006.*

The use of weighting has its own implications. Whilst it can be argued that it would provide more robust results statistically, the argument is dependent on a random sample being taken and the assumptions used holding true. The survey saw a large number of Spanish eggs sampled, almost three times the number that might have been expected if the import data was indeed a reflection of the proportion of non-UK eggs in the retail sector (see Annex G for HMRC / Defra import data). This is likely to be due to deficiencies in the first two assumptions made about the import data. If this is the case, then the true prevalence will likely be bounded above by the un-weighted *Salmonella* prevalence of non-UK eggs estimate and bounded below by the weighted estimate. It is not possible to determine with certainty which is the closer estimate, or indeed know for sure the un-weighted estimate is the upper boundary. The recommendation would be to use the weighted results, allowing for the fact that this may be lower than the true *Salmonella* prevalence in non-UK eggs. Confidence intervals were calculated for the prevalence rates where sample sizes were greater than 30. Statistical comparisons can not be made between factors. The majority of egg samples contaminated with *Salmonella* spp. were from Spain and there was insufficient data to account for the confounding effect Spain has. The results presented in this report are an account of the eggs sampled and may not reflect the overall non-UK egg population.

3.2 Collection and examination of non-UK egg samples in the survey

A total of 1,890 samples of six eggs were examined; each group of 6 eggs was pooled for testing. Of these, 145 of the pooled egg samples contained one or more eggs that were found to have visibly cracked shells and thus were considered as being of unacceptable condition and for this reason they were not included in the main data analysis but are presented in the annex (Annex H). One further sample did not have a country of origin stated and was also excluded from the analysis. Figures and proportions reported from this point forward are based on 1,744 pooled samples of acceptable 6 eggs rather than individual eggs.

3.2.1 Regional distribution of non-UK egg samples in the survey

Most of the non-UK egg samples were purchased in or around London (94.0%; n=1,639) with the remainder (6.0%; n=105) in north west England (Table 1), reflecting the availability of non-UK eggs on retail sale in these regions.

Non-UK eggs purchased from retail premises were produced in eight European countries (Table 1). Eggs from all eight countries were purchased in London while eggs from only four of these countries were bought in north west England. In proportion, the majority of the eggs produced in Spain were sampled from London retail premises (70.2%), while more eggs produced in Germany and The Netherlands were sampled from retail premises in north west England, 23.8% and 70.5%, respectively (Table 1). The lack of Spanish eggs in the north west of England was due to recent actions taken by a large importer to only source eggs from vaccinated flocks. Only non-UK eggs which were purchased in the London region were contaminated with *Salmonella* spp.

Table 1. Regional distribution of non-UK eggs purchased during the survey

Country of origin	Region				Total No. pools of eggs examined (%)	
	London	(%)	North West	(%)		(%)
Belgium	12	(0.7)	1	(0.9)	13	(0.8)
France	348	(21.2)	0	(0.0)	348	(20.0)
Germany	20	(1.2)	25	(23.8)	45	(2.6)
Poland	4	(0.2)	0	(0.0)	4	(0.2)
Portugal	25	(1.5)	0	(0.0)	25	(1.4)
Republic of Ireland	23	(1.4)	0	(0.0)	23	(1.3)
Spain	1,152	(70.3)	5	(4.8)	1,157	(66.3)
The Netherlands	55	(3.4)	74	(70.5)	129	(7.4)
Total	1,639	(100)	105	(100)	1,744	(100)

3.3 Prevalence of *Salmonella* in non-UK eggs at retail sale

Of the 1,744 samples of eggs included in the survey, 157 were positive for *Salmonella*. The most prevalent *Salmonella* serotype isolated was *S. Enteritidis* (2.6%; n=136), of which PT1 prevailed (2.2%; n=116). The estimated weighted

prevalence for all *Salmonella* spp. was 3.3% (Table 2). *Salmonella* Enteritidis PT4 was not isolated from any of the samples taken.

Table 2. Prevalence of *Salmonella* contamination in non-UK eggs (n=1,744 pools of eggs)

	Total No. of positive samples	Unweighted Prevalence rate (%)	Weighted Prevalence rate (%)	95% CI* (w) [†] (%)	
				Lower	Upper
S. Enteritidis	136	7.8	2.6	1.9	3.4
(<i>S. Enteritidis</i> PT1)	116	6.7	2.2	1.5	3.0
(<i>S. Enteritidis non-PT1</i>)	20	1.2	0.4		
Non-S. Enteritidis	21	1.2	0.8		
All <i>Salmonella</i> spp.	157	9.0	3.3	2.5	4.3

*, 95% confidence intervals

† (w) weighted confidence intervals

3.3.1 Prevalence of *Salmonella* spp., *S. Enteritidis*, *S. Enteritidis* PT1 and non-PT1 at 'box' level

The prevalence of all *Salmonella*, *S. Enteritidis*, *S. Enteritidis* PT1 and non-PT1 per sample of 6 eggs or 'box' is shown in Table 3. The *S. Enteritidis* and *S. Enteritidis* PT1 prevalence figures are equivalent to 1 in 40 and 1 in 50 boxes of 6 eggs, respectively.

Table 3. Estimated prevalence of *Salmonella* contamination per box of 6 non-UK eggs on retail sale (Figures rounded to nearest 10)

	Box Level Prevalence	95 % Confidence Intervals	
		Lower	Upper
All <i>Salmonella</i> spp.	1/30	1/40	1/20
<i>S. Enteritidis</i>	1/40	1/50	1/30
<i>S. Enteritidis</i> PT1	1/50	1/60	1/30
<i>S. Enteritidis non-PT1</i>	1/270	1/670	1/130
Non-S. Enteritidis	1/130	1/240	1/80

3.4 Country of origin in relation to presence of *Salmonella* spp.

Of the 1,744 pooled eggs sampled, most were produced in Spain (66.3%), France (20.0%) or The Netherlands (7.4%) (Table 4). *Salmonella* spp. were detected from 154 samples from Spain and 2 samples from France. Of the eggs produced in Poland one sample of the four boxes sampled tested positive for *Salmonella*. *Salmonella* was not recovered from eggs produced in Belgium, Germany, Portugal, Republic of Ireland or The Netherlands.

Table 4. Country of origin of non-UK egg samples and *Salmonella* positive pools

Country of origin	Total No. pools of eggs examined (n=1,744)* (%)	No. pools <i>Salmonella</i> positive (n=157)
Belgium	13 (0.7)	0
France	348 (20.0)	2
Germany	45 (2.6)	0
Poland	4 (0.2)	1
Portugal	25 (1.4)	0
Republic of Ireland	23 (1.3)	0
Spain	1,157 (66.3)	154
The Netherlands	129 (7.4)	0

*, each sample comprised 6 eggs

3.5 *Salmonella* isolated from both shells and contents - serotypes, phage types and antimicrobial susceptibility

At present there are no satisfactory sampling techniques that could effectively allow differentiation between *Salmonella* contamination on the shell and that in contents; thereby the method had the potential to provide false positive or negative results. The method used would give an indication as to the relative proportion of contamination on the shell or in the contents in all *Salmonella* contaminated eggs. Of the 157 samples positive, 10 were shell and contents positive. In six samples two separate salmonellas were detected making a total of 173 distinct isolates recovered from the shell and contents of acceptable non-UK egg samples (Table 5). The *Salmonella* isolates comprised eight different

serotypes, of which most were *S. Enteritidis* (84.9%; 147/173). Nine different phage types of *S. Enteritidis* were recovered, with PT1 predominating (82.9%; 122/147) (Table 5). The nine phage types identified amongst *S. Enteritidis* isolates included: PT1 (Nx_{Cp}_L; resistant to nalidixic acid with reduced susceptibility to ciprofloxacin), PT6a (A; resistant to ampicillin), PT6a (Nx_{Cp}_L), PT6d (A), PT7 (Nx_{Cp}_L), PT34, PT37 (Nx_{Cp}_L), PT53, PT56 and RDNC (Tables 5 and 6). Phage type 7 is a rough variant which can be derived from PT1 and PT4.

Table 5. *Salmonella* sero- and phage types recovered from non-UK egg shells and contents (n=173)*

<i>Salmonella</i> sero / phage types	No. Positive Shells Pools*	No. Positive Contents Pools	Total Positive Pools
<i>S. Enteritidis</i> (all types) [†]	140	7	147
<i>S. Enteritidis</i> PT1	116	6	122
<i>S. Enteritidis</i> PT6a	5	0	5
<i>S. Enteritidis</i> PT6d	1	0	1
<i>S. Enteritidis</i> PT7	7	0	7
<i>S. Enteritidis</i> PT34	2	0	2
<i>S. Enteritidis</i> PT37	1	0	1
<i>S. Enteritidis</i> PT53	1	0	1
<i>S. Enteritidis</i> PT56	5	1	6
<i>S. Enteritidis</i> RDNC [†]	2	0	2
<i>S. Braenderup</i>	1	0	1
<i>S. Infantis</i>	1	0	1
<i>S. Mbandaka</i>	11	3	14
<i>S. Panama</i>	1	0	1
<i>S. Rissen</i>	2	0	2
<i>S. Unnamed</i> (I O=Rough: g,m:-)	6	0	6
<i>S. Weltevreden</i>	1	0	1
Total	163	10	173

*, 6 samples had 2 separate *Salmonellas*

†, RDNC, reacted with the phage set but did not conform to a recognised phage type

The susceptibility of the 173 *Salmonella* isolates to the panel of 12 antimicrobial drugs is shown in Table 6. The majority of the *Salmonella* isolates were resistant to one or more of the antimicrobial drugs tested (83.2%; n=144), of which most were resistant to nalidixic acid with reduced susceptibility to ciprofloxacin (78.6%; n=136).

Table 6. *Salmonella* isolates recovered from the shell and contents of eggs produced in France, Poland and Spain (n=173)*

Country of Origin	No. Pools positive*	<i>Salmonella</i> sero / phage types and susceptibility to antimicrobials [‡]	No. Isolates
France	2	S. Mbandaka SuTTmNxCp _L	1
		S. Rissen ASuTTm	1
Poland	1	S. Braenderup ACp _L	1
Spain	154	S. Enteritidis (all types):	147
		PT1 (NxCp _L *)	120
		PT6a (A)	3
		PT6a (NxCp _L)	2
		PT6d (A)	1
		PT7 (NxCp _L)	9
		PT34	2
		PT37 (NxCp _L)	1
		PT53	1
		PT56	6
		RDNC [†]	2
		S. Infantis	1
		S. Mbandaka	13
		S. Panama	1
S. Rissen	1		
S. Unnamed [§] (NxCp _L)	4		
S. Unnamed [§]	2		
S. Weltevreden (ASSuSp _L TTm)	1		
Total			173

*, 6 samples had two separate salmonellas

†, RDNC, reacted with the phage set but did not conform to a recognised phage type

‡, Key to antimicrobials: A, Ampillicin; Cp_L, reduced susceptibility to ciprofloxacin; Nx, Nalidixic acid; S, Streptomycin; Su, Sulphonamides; Sp_L, reduced susceptibility to Spectinomycin; T, Tetracycline; Tm, Trimethoprim

§, I O=Rough: g,m:-

3.6 Sampling period in relation to country of origin and presence of *Salmonella* spp. in non-UK eggs at retail sale

Table 7 and Figure 1 illustrate the number of non-UK egg samples examined over the period of the survey. Eggs purchased during the months of January to May 2006 had the largest number of *Salmonella* present compared to eggs purchased in the other sampling months of the survey (n=154) (Table 7, Fig. 1).

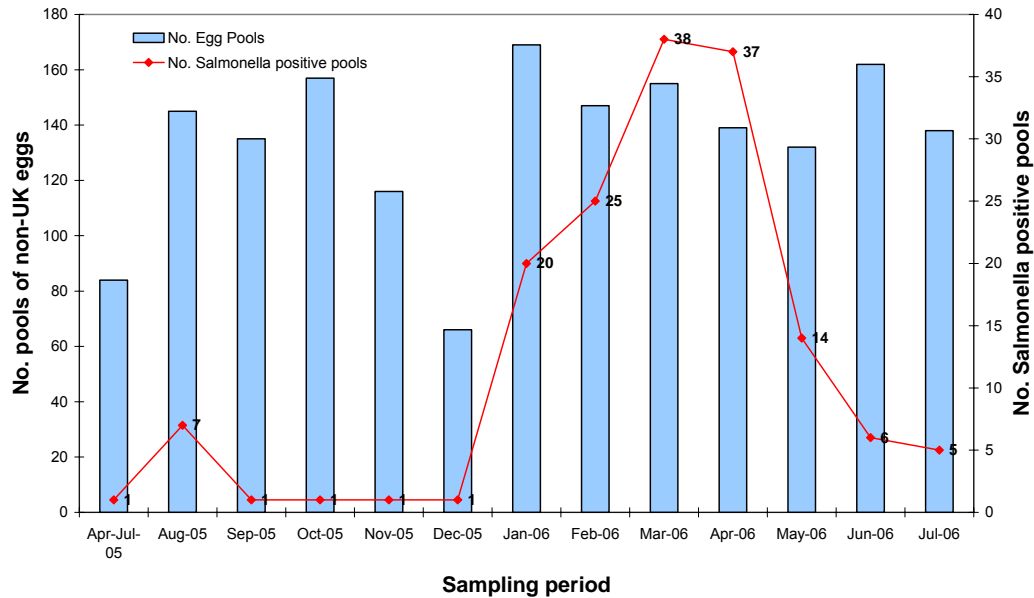
Of the 157 samples of eggs found to be contaminated with *Salmonella* spp., the majority (n=154) occurred during January to May 2006, of which, 151 were samples of eggs produced in Spain, two in France and one in Poland. Overall, *Salmonella* spp. was detected in 154 of 1,157 boxes of 6 or more eggs produced in Spain sampled from April 2005 to July 2006.

Table 7. Number of non-UK egg samples purchased during the sampling period and *Salmonella* positive pools

Year	Month	Total No. pools of eggs examined	No. pools <i>Salmonella</i> positive
2005	April* - June	66	0
	July	18	1
	August	145	7
	September	135	1
	October	157	1
	November	116	1
	December	66	1
2006	January	169	20
	February	147	25
	March	155	38
	April	139	37
	May	131	14
	June	162	6
	July	138	5
Total		1,744	157

*includes two days in March

Fig. 1 Sampling period in relation to presence of *Salmonella* spp. in non-UK eggs



* Apr- Jul data includes a small number of sampled taken during March.

3.7 Egg production details in relation to presence of *Salmonella* spp.

Over three-quarters (76.2%) of the eggs were labelled as cage, 23.6% as free range and 0.1% as barn produced. No organic eggs were sampled during the course of the survey (the market is very small and therefore was not represented during the random sampling). The production method for 0.1% of eggs was not available (Table 8). Egg sizes varied from small (0.5%), medium (54.4%), large (39.0%) to extra large (0.5%). The egg size was not available for 5.7% of samples (Table 8).

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) (<http://www.kat-datenbank.de/katdb/Index.do>). 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.

Table 8. Production details of non-UK egg samples and *Salmonella* positive pools

Egg details	Total No. pools of eggs examined (n=1,744)* (%)		No. pools <i>Salmonella</i> positive (n=157)
Production type			
Barn	2	(0.1)	0
Cage	1,329	(76.2)	132
Free range	411	(23.6)	25
Not Stated†	2	(0.1)	0
Egg size			
Small (<53g)	8	(0.5)	0
Medium (53g - ≤ 63g)	948	(54.4)	55
Large (63g - ≤ 73g)	680	(39.0)	95
Extra large (≥ 73g)	9	(0.5)	3
Not Marked‡	99	(5.7)	4
Pack size			
6	1,043	(59.8)	99
10	385	(22.1)	46
12	65	(3.7)	3
18	117	(6.7)	5
24	7	(0.4)	0
30	43	(2.5)	0
Tray eggs (30)	63	(3.6)	4
Tray eggs (36)	18	(1.0)	0
Box eggs (360)	3	(0.2)	0
Appearance of eggs			
Clean	336	(19.3)	5
Dirty	1,403	(80.3)	152
Not Stated	5	(0.3)	0

*, each sample comprised 6 eggs

†, No stamp mark on egg shells

‡, Size not stated at the point of sale or marked on the box

3.7.1 Condition of non-UK eggs in relation to production details and retail premises type

During the course of the survey the laboratory staff made a subjective decision on whether or not an egg was dirty (Annex D). If one egg within a group of eggs had visible signs of contamination then the whole group would be described as dirty. Through this classification process, the number of dirty eggs reported in this survey may be higher than what is actually occurring in the non-UK egg market.

Most (80.3%) of the egg shells in the pooled samples were recorded as having a dirty appearance, i.e. visibly soiled and/or presence of hen faeces (Table 8). There is a degree of tolerance allowed for Grade A eggs and this is covered in Regulation (EC) 2295/2003, Article 33 (EC 2003b). At packing centres, Egg Marketing Inspectors (EMIs) may downgrade if quality faults exceed 5% of the sample checked from any single batch. At any other marketing stage (e.g. wholesale, retail, catering premises) an additional 2%, taking the tolerance up to 7% total, is permitted before EMIs may downgrade eggs.

In broad terms approximately 1 in 3 eggs may have some slight faecal or similar dirt upon the shell, but this is normally insignificant. EMIs have to make a subjective judgement to satisfy themselves that an egg is sufficiently dirty to be deemed a quality fault. This subjective judgement is based upon the amount and type of contamination on the shell (personal communication - EMI).

3.8 Retail premises details in relation to presence of *Salmonella* spp.

Retail signage or receipt was used to categorise the premises visited during the course of the survey. Convenience stores (44.3%) and mini-supermarkets (33.5%) formed over three-quarters of the premises visited (Table 9). Supermarkets (13.1%), butcher shops (4.8%) and other premises (delicatessen, post office, service station, wholesalers, greengrocers, market shop/stalls and newsagents) were also visited (4.3%) (Table 9). *Salmonella* spp. were detected from non-UK eggs purchased from all types of retail premises visited.

Table 9. Retail premises details where non-UK egg samples were purchased and *Salmonella* positive pools (n=1,744)

Premises details	Total No. pools of eggs examined (n=1,744)* (%)		No. pools <i>Salmonella</i> positive (n=157) (%)	
Premises type				
Butcher shop	83	(4.8)	6	(7.2)
Convenience store	773	(44.3)	70	(9.1)
Mini-supermarket	583	(33.5)	60	(10.2)
Supermarket	230	(13.1)	16	(7.0)
Other [†]	75	(4.3)	5	(20.0)

*, each sample comprised 6 eggs

†, Delicatessen, post office, service station, wholesaler, greengrocers, market shop/stalls, newsagents

3.9 Shelf-life of non-UK egg samples in relation to presence of *Salmonella* spp.

The best before date of eggs must not exceed 28 days from the date of lay (EC 2003b, Annex D). The date was available for 93.6% (1,633) of non-UK eggs sampled (Table 10). For each of these samples, the number of days remaining until consumption (i.e. the number of days between the date sampled and the best before date) was determined and the results are provided in Table 10. It is usual that non-UK eggs take approximately 3-5 days from producer to reach retailer; therefore it is assumed that no more than 24 days can be available before the expiration of the best before date (BBD).

Most samples had between 0 and 24 days remaining before the BBD (97.9%). Regarding the proportion of samples in breach of the sell by date (to be sold by 21 days from lay) and BBD requirements (i.e. must not exceed 28 days from lay), 23.3% had 7 or less days and 0.9% had in excess of 25 days remaining before the BBD expired. A small proportion of eggs samples (1.2%) were purchased after their best before date. These and those with a BBD assigned greater than 28 days are in breach of Regulation (EC) No. 2295/2003 (Annex D) (EC 2003b).

Table 10. Remaining days until expiration of best before date of non-UK egg samples and *Salmonella* positive pools

Egg details	Total No. pools of eggs examined (n=1,744)* (%)		No. pools <i>Salmonella</i> positive (n=157)
Best before date			
Marked	1,633	(93.6)	153
Not marked [†]	109	(6.3)	4
Illegible or invalid	2	(0.1)	0
Remaining days until best before date (n=1,633)			
>28 [‡]	2	(0.1)	0
25-28 [‡]	13	(0.8)	0
22-24	31	(1.9)	2
15-21	535	(32.8)	58
8-14	653	(40.0)	60
0-7 [‡]	380	(23.3)	31
<0 [‡] (expired)	19	(1.2)	1

*, each sample comprised 6 eggs

†, No best before date marked on egg shells or on the box

‡, In possible breach of the BBD or SBD requirements

3.9.1 Storage temperature of eggs at retail premises

Information of the storage conditions were collected at the point of purchase. Where recorded, this ranged from 10°C to 27°C for ambient stored eggs. The majority of the eggs sampled were stored at ambient temperature (99.2%; 1,731/1,744) with a few stored at chilled (0.8%; 13/1,744). No comparisons could be made between storage temperatures due to the large difference in numbers sampled. *Salmonella* spp. were only recovered from eggs stored at ambient temperature (9.1%, 157 /1,731).

There are no egg regulations which state that raw shell eggs need to be stored in chilled cabinets at retail. Storage at stable ambient temperatures (eg 18-20°C) in retail outlets helps keep the temperature fluctuations to a minimum. By storing the eggs at ambient temperature the formation of condensation on the egg shell once the eggs have been purchased can be prevented. It might be difficult to

maintain the cold chain once the consumer purchases the eggs from any retailer. However, it should be noted that where ambient temperatures exceed 20°C, *Salmonella* is capable of surviving and multiplying (Cogan *et al.* 2003).

3.10 Traceability of non-UK eggs sampled at retail premises

The majority of samples (99.5%) had a stamp mark on the egg shell. Four (0.2%) samples were unmarked. Stamp mark details were not recorded for five samples (0.3%) (Table 11). All 157 samples that had *Salmonella* spp. present were from non-UK eggs that had stamp mark details on the egg shell (157 of 1,735) of which there were 26 different stamp marks.

In 18 samples, individual eggs were marked with different stamp marks (2-4 stamp marks in a sample of 6 eggs, suggesting supply from multiple producers to the packing station) but all related to the same country of origin that was France (n=4), Germany (n=1), The Netherlands (n=1) or Spain (n=12). Five samples of non-UK eggs with stamp marks had two or more of individual eggs with part of the stamp mark illegible, i.e. digits comprising some of the code of the farm where the eggs were produced were indecipherable. Additionally, in 47 of egg samples not all the individual eggs were stamp marked in the sample of six eggs and these originated from France (n=13), The Netherlands (n=6), Poland (n=1), Republic of Ireland (n=2) and Spain (n=25).

Similarly, most samples (91.3%; n=1,593) had a packing station number available. Non-UK egg samples contaminated with *Salmonella* spp. were associated with nine packing station numbers, of which the majority (82.1%; n=129) were linked with one packing station number (ES1400116/VA) supplied by three farms (1ES4721421, 3ES4721431 and 3ES4721441).

Table 11: Traceability details of non-UK egg samples

Egg details	Total No. pools of eggs examined (n=1,744)* (%)		No. pools <i>Salmonella</i> positive (n=157)
Stamp mark on shells			
Marked	1,735	(99.5)	157
Not marked	4	(0.2)	0
Not recorded	5	(0.3)	0
Packing station number			
Marked on box	1,593	(91.3)	152
Not marked on box	151	(8.7)	5

*, each sample comprised 6 eggs

4. Discussion

Salmonella contamination of eggs has been one of the main microbiological food safety issues in the last 20 years, with outbreaks of *S. Enteritidis* infection associated with raw shell eggs continuing to be a common cause of food borne illness (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). The consumption level of eggs in the UK exceeds the national supply, resulting in the need to source eggs from outside the UK (Defra 2006).

Statistical considerations of the scope of the survey

At the outset of this survey, market intelligence suggested that non-UK eggs were primarily available in two UK regions: the north-west of England and London. Therefore, the survey sampled from these two regions. Due to public health initiatives regarding sourcing eggs in the north-west of England (i.e. sourcing eggs from only vaccinated layer flocks) (HPA 2004, 2005a), the majority of the samples came from London as there was limited availability of non-UK eggs in the north west. Though it is believed that the survey reflects the availability of non-UK eggs on retail sale however, it cannot be said that the survey is representative as there is no conclusive evidence to show that non-UK eggs are not sold elsewhere in the UK. Scoping exercises in other geographical locations were carried out during the survey. The Agency also issued a news item in Environmental Health News in October 2005, requesting EHO's to provide details of any retail outlets selling non-UK eggs (FSA 2005c). Non-UK eggs could not be sourced at retail in other locations from these exercises.

At present, there is no information detailing every single UK retail premises that sells non-UK eggs or any eggs for that matter. As such, it cannot be guaranteed that the survey is random and so the results are likely to be biased in some way. Given the potential biases it is not recommended that comparisons be made from this survey with the UK produced egg retail survey carried out in 2003 (FSA 2004a). The time periods for these two surveys are also not comparable; the

non-UK egg survey collected eggs over a period of 16 months, whilst the UK produced egg survey was over a period of three months.

Additionally, the results of this survey are not comparable to the Department of Health (DH) non-UK egg survey carried out in 1996/97 (ACMSF 2001). The *Salmonella* prevalence estimates obtained from that survey were not weighted and so were not representative of the non-UK egg population. Coupled with the difference in methodologies between the two surveys means that it is not possible to make such comparisons.

The weighting used in the current survey is unlikely to account for all the potential bias introduced by the sampling plan (see paragraph 3.1). The main potential source of bias is the lack of a sampling frame. As the locations of all the retailers that stocked non-UK eggs were not known, it is plausible that bias was introduced via the retailers selected. The use of the non-UK egg import data to weight the results may have gone some way to address these biases but the data itself was for eggs at import level and this may not reflect the non-UK eggs that are on retail sale.

Given the lack of sample frame and therefore the lack of randomness in the selection of premises, likely bias introduced and inadequate information to correct this, the results from the survey are not statistically robust to make generalisations about the *Salmonella* prevalence in the non-UK egg population. Indeed, care needs to be taken when discussing the results obtained from the survey. The results should be used as an indication only of what may be occurring in the non-UK egg population.

However, despite all of the riders outlined in the discussion sections, the prevalence of *Salmonella* contaminated eggs found in this survey are uncomfortable high. The FSA should look to some of its major trading partners to take positive action to reduce the levels of *Salmonella* found in its eggs.

Overall results of the survey

Surveys of non-UK eggs were previously carried out in 1991 (de Louvois 1993) and 1996/97 (ACMSF 2001), although these were not retail surveys as most non-UK eggs are supplied to the catering and/or manufacturing sectors. This is the first survey to provide information on *Salmonella* contamination of non-UK eggs on retail sale. Based on a survey of 1,744 acceptable samples of non-UK eggs purchased in London and the north west of England during 2005/06, the total weighted prevalence estimate for all *Salmonella* spp. detected in non-UK eggs was 3.3%. However, the true prevalence will likely be bounded above by the un-weighted *Salmonella* prevalence of non-UK eggs estimate of 9.0% and bounded below by the weighted estimate of 3.3%. Of the eggs sampled, most were produced in Spain (66.3%), France (20.0%) or The Netherlands (7.4%). *Salmonella* spp. was detected from 13.3% and 0.6% of eggs sampled that were produced in Spain and France, respectively.

Eight different *Salmonella* serotypes were recovered from non-UK eggs, of which *S. Enteritidis* predominated with an estimated prevalence of 2.6%. Moreover, *Salmonella* Enteritidis was only obtained from Spanish eggs. Nine different phage types of *S. Enteritidis* were identified; *S. Enteritidis* PT4 was not detected, whilst PT1 was found to be the predominant phage type (2.2%). *Salmonella* Enteritidis PT1 until now had not been detected in eggs examined as part of previous UK egg surveys (de Louvois 1993, ACMSF 2001) but has been detected in eggs of Spanish origin examined during the national outbreaks of *S. Enteritidis* non-PT4 infections in England and Wales from 2002 to 2004 (HPA 2004, Little *et al.* 2006). The greatest increases of human *S. Enteritidis* non-PT4 infections observed over this period was in *S. Enteritidis* PT1 and PT14b (HPA 2004, 2005a). Similarly in Spain during 2002 to 2003, *S. Enteritidis* PT1 was the most frequent type implicated with foodborne disease outbreaks associated with eggs and eggs products (Crespo *et al.* 2005). It is possible that in Spain phage types other than PT4, such as PT1, are replacing the biological niche previously occupied by PT4, although this requires verification.

Most of the *S. Enteritidis* isolates obtained from Spanish eggs in the present study were resistant to nalidixic acid with concomitant decreased susceptibility to ciprofloxacin (NxCP_L), (0.125 – 1.0 mg/l), or ampicillin. These resistance profiles were also found in *S. Enteritidis* isolates from Spanish eggs associated with multiple common source outbreaks of *S. Enteritidis* infection during 2002 to 2004 (HPA 2004, Little *et al.* 2006). Previous egg surveys have shown that antimicrobial resistance is uncommon in isolates of *S. Enteritidis* from UK-produced eggs (de Louvois 1993, Elson *et al.* 2005, FSA 2004a). It has been suggested that the use of quinolones in poultry has led to the emergence of *Salmonella* spp. that are resistant to older style quinolone antimicrobials, such as nalidixic acid, and with concomitant reduced susceptibility to fluoroquinolone antibiotics, for example ciprofloxacin (EFSA 2004). However, antimicrobial prescribing in human medicine has probably contributed the most to the development of resistant bacteria (ACMSF 1999). Rates of quinolone resistance in bacterial isolates from agricultural animals in Spain and Portugal have been shown to be higher than in the UK (Engberg *et al.* 2001). The consequences of bacterial resistance to certain antimicrobials, particularly fluoroquinolones, are of increasing public health concern since these are critically important for the treatment of severe *Salmonella* infections in humans (EFSA 2004, SCVPH 2003, Threlfall *et al.* 2003). *Salmonella* infection however usually causes a self-limiting diarrhoeal illness where treatment is usually limited to rehydration and rest (ACMSF 2001). A recent EFSA Opinion recommended that the use of antimicrobial for *Salmonella* control in poultry should be discouraged due to public health risks associated with the development, selection and spread of antimicrobial resistance (EFSA 2004). Consequently as part of further EU harmonised rules on *Salmonella* control measures in poultry implemented in 2006, antimicrobials should not be used except under very limited circumstances, such as animal health and welfare grounds (EC 2006b).

In most of the contaminated egg samples, *Salmonella* was found to be on, rather than in, the egg; out of the 157 *Salmonella* positive shell samples, 10 of these

were also contents positive. There is an obvious cross-contamination risk from this external contamination, in that the organism can be transferred to other surfaces (e.g. hands) or to the egg contents during breaking of the shells. The presence of *Salmonella* on the surface of the shells of eggs therefore represents a potential risk to public health, just as well as contamination of the contents of the egg. Surface contamination may be the result of either infection of the lower reproductive tract of hens or faecal contamination (de Buck *et al.* 2004, Humphrey 1994). Reduction of this risk requires measures to be implemented at farms and packing stations to further reduce the prevalence of contamination and precautions are required by retailers and consumers to minimise spread, including adequate cooking of eggs and prevention of cross-contamination.

Class A eggs must be sold to the consumer within a maximum time limit of 21 days from the date of lay in order that the consumer may have sufficient domestic use of the product before the 'best before' date (28 days from date of lay) expires. Any eggs remaining on the shelves for retail sale after 21 days from date of lay should be removed (EC 2003b, MAFF 1996a). Approximately a quarter (25.3% 414/1,633) of non-UK eggs purchased from retail premises were found to have either expired best before dates or were still on sale after 21 days from date of lay.

Class A eggs must also be of a clean and undamaged appearance (EC 2003b). With regard to dirty eggs and egg inspections, it should be noted that approximately 1 in 3 eggs may have some slight faecal or similar dirt upon the shell, but this is normally insignificant. Egg Marketing Inspectors (EMIs) have to make a subjective judgement to satisfy themselves that an egg is sufficiently dirty to be deemed a quality fault. This subjective judgement is based upon the amount and type of contamination on shell (personal communication, EMI). A large proportion of retail non-UK eggs were found to have an unclean and/or cracked appearance. These potential breaches of the egg marketing regulations ((EC) No 2295/2003) were categorised by the laboratory staff and were reported

to the UK Egg Marketing Inspectorate via the FSA to take action where appropriate.

Non-UK eggs make up a relatively small but growing proportion of the UK market with The Netherlands, Germany and Spain being the major exporters of eggs to the UK (Defra 2006). Non-UK egg samples contaminated with *Salmonella* spp. were associated with nine packing stations, of which most (82.1%) were linked with one packing station in Spain. The presence of *Salmonella* contamination in egg-packing stations presents a risk of cross-contamination. Contamination of egg packing stations does occur and transfer of this contamination to the external surface of shell eggs is possible (Davies and Breslin 2003). What is unclear, however, is whether there is a reduction in the numbers of *Salmonella* on eggs during the grading and packing process. In addition to control of *Salmonella* infection at the flock level, Davies and Breslin (2003) identified that improved methods of cleaning and disinfecting egg-handling equipment at egg packing stations were required.

It was evident that the majority of the *Salmonella* positives (82.1%; n=126) were linked with one particular packing station ES1400116/VA (also recorded as 111400116/VA) which appears to source its eggs from 3 producers (ES4721421, ES4721431 and ES4721441). The high number of positives from these specific producers could indicate problems of contamination within the laying flock or cross contamination at the packing station.

A small proportion (5.9%) of samples were produced from laying hens vaccinated against *Salmonella* or under a controlled assurance scheme, none of which were contaminated with *Salmonella*. In the WHO/FAO risk assessment of *S. Enteritidis* in eggs, reducing the prevalence of *S. Enteritidis* in poultry flocks was directly proportional to the reduction in risk to human health (WHO/FAO 2002). Vaccination of layer flocks, or those certified as free from *S. Enteritidis* and Typhimurium under controlled assurance schemes, combined with improved

biosecurity, does appear to have had a significant impact on the prevalence of *S. 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 as demonstrated by the recent upsurge in *S. Enteritidis* non-PT4 infections in European countries (Fisher 2004).

To achieve sustained decreases in egg-associated *S. Enteritidis* infections, a concerted effort is needed from farmers to consumers. A key factor in this effort is the implementation of farm-based controls to reduce *S. Enteritidis* contamination of eggs during production (EC 2006a, 2006b, EFSA 2006). An EU-wide *Salmonella* baseline study on commercial laying hen holdings or farms during 2004 to 2005 has quantified the levels of *Salmonella* in the environment of the holdings. The overall observed *Salmonella* holding prevalence was 30.7%, although there was a wide variation in observed holding prevalence between Member States, ranging from 0% to 79.5%. The *Salmonella* observed holding prevalence was highest in Portugal (79.5%), Poland (77.2%) and Spain (73.2%). *Salmonella* Enteritidis was the most frequently isolated serotype from laying hen holdings, although holdings having *S. Enteritidis* vaccinated flocks were less likely to be positive for *S. Enteritidis* (EFSA 2006).

In light of these findings, targets have been introduced for the reduction of *Salmonella* in laying hens, which in turn should lead to a lower prevalence of *Salmonella* in eggs (EC 2006a). Every Member State will have to work towards reducing the number of laying hen holdings contaminated with *Salmonella* by a specific minimum percentage each year, with steeper targets for Member States with higher levels of *Salmonella*. The first target deadline is set for 2008. There will also be mandatory vaccination of layer hens against *Salmonella* from 2008 onwards for layer hen holdings in Member States with a *Salmonella* prevalence of 10% or more (EC 2006b).

Impact of the survey findings on Food Standard Agency (FSA) advice

The FSA's current advice to at risk consumers is that cooking eggs properly will kill any vegetative 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:

[\(http://www.eatwell.gov.uk/healthydiet/nutritionessentials/eggsandpulses/eggs/\)](http://www.eatwell.gov.uk/healthydiet/nutritionessentials/eggsandpulses/eggs/).

The FSA has also issued a leaflet for caterers on the use of eggs (<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 FSA will consider the results of this survey along with those of other surveys (such as the current survey on *Salmonella* contamination of raw shell eggs used by catering premises) and the results of available research when considering its advice to consumers on egg consumption.

5. Conclusions

The estimated prevalence of all *Salmonella* spp. and *S. Enteritidis* was reported to be from 3.3% and 2.6%, respectively from non-UK eggs sold on retail sale in London and the north west of England.

Of the eggs sampled, most originated from Spain, with substantial numbers also sampled from France and The Netherlands. *Salmonella* spp. was detected from 13.3% and 0.6% of eggs sampled that were produced in Spain and France, respectively.

A substantial number of *Salmonella* contaminated eggs were associated with one egg packing station in Spain (129 out of 154 contaminated samples), in turn supplied by three egg producing farms. This may suggest that *Salmonella* contamination occurred at the layer flock farms and/or at the egg-packing station by cross-contamination from egg handling equipment.

Salmonella Enteritidis was only recovered from Spanish eggs. Although nine different phage types of *S. Enteritidis* were recovered, no PT4 isolates were detected, whilst *S. Enteritidis* PT1 was found to be the predominant phage type (2.2%). This may suggest phage types, such as PT1, are replacing the biological niche once occupied by PT4 in Spain.

Most of the *S. Enteritidis* isolates obtained from Spanish eggs in the present study were resistant to nalidixic acid with concomitant decreased susceptibility to ciprofloxacin (NxCp_L) or ampicillin. It has been suggested that the reduced susceptibility to ciprofloxacin in *S. Enteritidis* isolates obtained from Spanish eggs may be a consequence of the use of fluoroquinolone antibiotics in poultry (EFSA 2004).

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Annexes to the Report of the Survey of *Salmonella* Contamination of Non-UK Produced Raw Shell Eggs on Retail Sale

**ANNEX A: FOOD STANDARDS AGENCY SURVEY OF *SALMONELLA*
CONTAMINATION OF NON-UK PRODUCED SHELL EGGS ON RETAIL SALE:
PROTOCOL**



SURVEY OF *SALMONELLA* CONTAMINATION
OF NON-UK PRODUCED SHELL EGGS ON
RETAIL SALE

PROTOCOL

CONTENTS

OUTLINE	3
▪ Background	3
▪ Objectives	4
▪ Publication of results	4
▪ Timetable	4
SURVEY DESIGN	5
SAMPLING	6
▪ Sample collection	6
▪ Sample information	6
TESTING	8
▪ Receipt of samples	8
▪ Examination	8
▪ Salmonella methodology	8
▪ Data handling and reporting	9
▪ Quality assurance	9
▪ Zoonoses Order	10
APPENDICES	11
1. Timetable	11
2. Sampling plan	12
3. Definition of Class A eggs and egg stamping requirements	13
4. Laboratory methodology	14

OUTLINE

Background

1. Between 1981-1991 the number of cases of salmonellosis in the UK in humans rose by approximately 170%. 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 pandemic. The *Salmonella* in eggs working group 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 a chicken.¹ This work coincided with the publication of a Department of Health (DH) funded survey² of the prevalence of *Salmonella* contamination of individual eggs from high street retailers, which indicated a contamination rate of 1 in 108 boxes of 6 eggs on either the shell and/or in the contents in UK eggs.
2. A further survey on UK produced eggs in 1995/96 suggested little improvement, with *Salmonella* being present in 1 in 101 boxes of 6 eggs.³ However, a more recent survey in 2003 of UK eggs on retail sale showed a reduction in *Salmonella* contamination to 1 in 290 boxes of 6 eggs.⁴ This has been accompanied by a large decrease in the number of cases of human infection caused by *Salmonella* Enteritidis PT4.
3. No data is available on *Salmonella* contamination of non-UK eggs on retail sale. In 1991 a DH funded survey of imported eggs at the port of entry was carried out by the PHLS. The *Salmonella* prevalence was 1.6% from 8,630 samples of six eggs. Out of the total number of Salmonellas, 13.8% were *S. Enteritidis* and 11.6% were *S. Enteritidis* PT4. In 1996/97, the Department of Health (DH) carried out a further survey, comprising of 1,433 samples of 6 non-UK shell eggs taken from importers. The study found the prevalence of *Salmonella* in non-UK eggs to be 1 in 49 boxes of 6 eggs. The survey found that the total *Salmonella* prevalence was 2.0%. Out of the total number of salmonellas, 62% were *S. Enteritidis* and 7% were *S. Enteritidis* PT4. In two of the samples *Salmonella* was isolated from the shell and contents. The results from these surveys suggest that there had been little improvement in *Salmonella* contamination of these non-UK eggs.

¹ Advisory Committee on the Microbiological Safety of Food (1993). Report on *Salmonella* in eggs. HMSO, London.

² De Louvois, J. (1993). *Salmonella* contamination of eggs. Lancet; **324**: 366-367.

³ Advisory Committee on the Microbiological Safety of Food (2001). Second report on *Salmonella* in eggs. HMSO, London.

⁴ Food Standards Agency Report of the survey of salmonella contamination of UK produced shell eggs on retail sale. March 2004

Objectives

4. The primary objective of the survey is to determine the level of *Salmonella* contamination of non-UK produced shell eggs on retail sale.
5. It should be noted that this survey focuses on non-UK produced eggs and is not intended to provide information on seasonality. Samples will be taken over the course of a year to reflect change in the sourcing of non-UK eggs over that time. As far as possible, the aim will be to identify the country of origin of all eggs tested and the source of all *Salmonella* positive eggs. Production types of egg will be recorded, however, the survey will not be able to collect enough samples of less common production types to make statistical comparisons

Publication of results

6. The FSA has a policy of openness and releases all details associated with samples analysed in its surveys including brand names, packing station codes and the company names and addresses of retail shops. The Agency will not withhold further information collected in association with this survey if after due consideration it considers this to be in the public interest. At the end of the survey, the results and all the information that has been collected about the samples will be published on the Agency's website.

Timetable

7. A proposed timetable is available at Annex 1.

SURVEY DESIGN

8. The main objective of the survey is to determine the level of *Salmonella* contamination of non-UK produced eggs. The number of eggs required to be tested to achieve this objective depends on the degree to which the rate of contamination has changed. Since the degree to which the rate has changed is unknown, the decision has been taken to start by testing 2,500 samples in the course of a year. The results will be assessed and if the sample is not large enough to demonstrate a statistically significant change, sampling will be extended.
9. The contractor will be responsible for ensuring that the appropriate number of samples (as outlined in Annex 2) are collected. Seasonal effects are not being investigated; therefore it is acceptable for the contractor to cover retail outlets region by region rather than continually sampling throughout the UK. Retail outlets should not be sampled more than once and no more than 4 samples should be collected from any one store. However, retail outlets can be revisited after a period of 4 weeks, to ensure different batches of eggs are sampled. If the samplers are unsure of the production type or country of origin, the eggs should not be sampled.
10. The aim of this survey is to sample 2,500 boxes of 6 eggs. The number of samples should be reviewed by the Agency every 3 months to ensure that enough eggs are being sampled to meet the required quota.
11. Retailers should receive payment for the eggs at the time of sampling. The contractors will be responsible for organising this payment.
12. Contractors should provide smaller independent retail outlets (i.e. farm stores, market, stalls, milkmen and butchers) with a letter from the Agency informing them that samples have been taken from their premises in order to carry out a survey. For larger retail chains this may not necessary as the relevant contact at head office will be sent a list of their premises from which samples have been obtained.

SAMPLING

Sample collection

13. It is essential that cross-contamination is avoided during collection of eggs. Precautions must therefore be taken at all stages to ensure that the equipment used during sampling, transport and storage is not contaminated with *Salmonella*.
14. Contractors should aim to collect samples at random from the shelves and not necessarily at the front of the display. As there is no available scientific evidence that there is an association between size of egg and prevalence of *Salmonella* there is no need for samplers to select specific sizes. The temperature of the eggs should be recorded, as should information on whether they were displayed in a temperature controlled environment e.g ambient or chilled cabinet
15. Non-UK eggs sold at retail level must be of Class A quality (see Annex 3 for definition of Grade A egg). Should the contractor find any egg sampled to be in breach of the Class A standards they should report findings to the FSA on an informal basis who will contact the Egg Marketing Inspectorate for further investigations. The contractor shall inform the samplers to informally report such eggs to Trading Standards Officers (TSO's) for further investigation or action. Samples must consist of intact eggs with no evidence of damage. A brief examination of each box of eggs, without touching the contents, should be possible to eliminate any grossly damaged eggs. Each box must then be placed in a separate sampling bag to avoid the risk of cross contamination during transport and storage. The preference should be for sampling of boxes of 6 eggs but larger boxes may be sampled if that is all that is on sale and the extra eggs discarded at the laboratory.
16. At some retail outlets (e.g. market stalls or farm shops) it may not be possible to buy pre-packaged eggs and retailers may pack boxes of eggs from larger trays on demand. These should be examined as above. Samplers should also make certain that the eggs collected are non-UK produced.
17. Contractors should set in place a contingency plan to ensure enough samples are collected (e.g. to allow for any eggs broken in transit).
18. Contractors should ensure that samples are kept at approximately 20°C during transportation. Samples should be kept dry and out of direct sunlight in a cool-box. A calibrated data logger should be placed with the samples to monitor compliance with these requirements to ensure that samples were not stored above 25°C.

Sample information

19. All relevant information available from the sample should be entered onto a sampling form. As far as possible this information should include date and time of purchase, retailer property number, street name and postcode, packing station code, egg stamped code (to include country, production type and farm code), size, best before date, price and brand name (sell by date and packing date should also be recorded if available). The contractor will also enter this data onto an Excel 97 spreadsheet.

20. Each sample should be given a unique identification number that includes letters (e.g. EF2000 England, Free range) in order to clearly link to a particular sample. This reference number should be retained throughout testing and when any isolates are sent for typing.

TESTING

Receipt of samples

21. On receipt of the samples, laboratories should complete a laboratory record form to record details of LIMS number, appearance, country code, production code, farm code, organic reference number, address of packer / producer, sell by date and packing date if available etc.
22. A digital photograph of high resolution should be taken of the packaging and stored on a CD-ROM as a JPEG file under the appropriate sample number. The photograph should be of a high resolution so that the labelling details are clear. If this is not possible the contractor must retain all packaging (or a photocopy/photograph of it).
23. Eggs should reach the laboratory within 24 hours of sampling. Eggs should be held at approximately 20°C and the laboratory sampling procedure should begin within 24 hours of arrival at the laboratory. Eggs should always be tested before their best before dates.

Examination

24. Eggs should be examined visually before testing to confirm the absence of cracks and if any marks (e.g. faecal material, dust, mud, blood and the contents of other eggs) other than natural markings or printed marks are present, the eggs will be considered dirty. If any egg from a box of 6 is discovered to be cracked the entire box should be discarded and one of the contingency samples used in its place.
25. It is essential that handlers take care to avoid cross contamination between boxes and from the surrounding environment at all stages (e.g. by wearing gloves and changing them between each box of six eggs). Thorough cleaning of equipment and work surfaces must be undertaken regularly. There must be environmental sampling of the laboratory for *Salmonella* prior to and after to egg testing. The contractor should carry out examinations in areas dedicated to the examination of survey samples and clearly separated from potentially contaminated materials such as meat, chicken or faecal samples. It is also important that the laboratory control strain should be a rare serotype of *Salmonella* not associated with the contamination of eggs.

Salmonella methodology

26. The methodology for *Salmonella* testing is set out in Annex 4. The method used is similar to that of the last survey in which shell and contents were tested separately. However, it should be noted that there is no method

currently in existence that satisfactorily distinguishes shell from content contamination and the result will therefore be reported as a rate per group of six eggs as in the previous survey. However, separate data for shell and contents contamination will be included as an annex in the report.

Data handling and reporting

27. At monthly intervals the contractor will submit to the Agency a summary report containing details of the samples collected to date.
28. An interim report (electronic and hard copy) containing a summary of the results to date will be submitted to the Agency at bi-monthly intervals. The contractor will be expected to incorporate into their spreadsheet data on serotyping and phage typing for any *Salmonella* isolates sent for typing.
29. The contractor is responsible for collating all the results and a final report will be submitted to the Agency once the survey has been completed. The report will present summary statistics on the prevalence of *Salmonella*, together with a breakdown of the serotype and phage types. The results should not be subjected to detailed statistical analysis by the contractor as this will be undertaken by the Agency's statisticians.
30. All forms, documentation and electronic files must be retained by the contractor until further notice from the Agency in case of issues arising after completion of the survey. It is not necessary to provide the FSA with hard copies of forms. However, this information must be available to the FSA if required.

Quality assurance

31. In order to ensure a high level of accuracy in data entry, checking and back up of information must be accredited by an appropriate organisation (e.g. UKAS). The contractor must also be able to demonstrate satisfactory performance in the testing of food for *Salmonella* through participation in an external proficiency testing scheme (e.g. HPA, FEPAS). This should include the testing of External Quality Assessment samples by a different technician on three occasions throughout the survey. The FSA will visit the contractors during the course of the survey to assess how the work is being carried out.

Zoonoses Order 1989

32. Under the Zoonoses Order 1989, laboratories, which isolate *Salmonella* from foodstuffs, must provide Defra with a listing of subtype found together with the name of the retailer where the eggs were purchased.

ANNEXES

ANNEX 1: PROPOSED TIMETABLE FOR SURVEY

22 November 2004	Revised protocol out to consultation
Early December 2004	Meeting with importers
2 December 2004	Protocol finalised
17 December 2004	Contractor finalised for survey
21 February 2005	Contract signed
01 March 2005	Contract and sampling starts
01 May 2005	First interim report
01 April 2006	End of sampling and testing period
01 May 2006	Final report received
Late Summer 2006	Final report published

ANNEX 2: SAMPLING PLAN

A sample size of approximately 2,500 eggs is needed to achieve the precision required. The sample numbers should be reviewed periodically to ensure that statistically significant analyses could be carried out.

This is based on the following:

Assume that the prevalence estimate in the survey comes out at 1.5%. A 95% confidence interval on this estimate a range from a fraction over 1% to a fraction above 2%; i.e. we would have high confidence that the true prevalence lies in the range (1% to 2%).

ANNEX 3: DEFINITION OF A CLASS A EGG AND EGG STAMPING REQUIREMENTS

The regulations specify the minimum characteristic for quality classes. Class A eggs must have a normal shell cuticle which is clean and undamaged, the egg must be free of foreign smells and wet or dry clean is not permitted. Eggs sold at retail outlets should be of Class A quality. A 28 day best before date is the legal maximum in order for the egg to retain its Class A qualities. It should also noted that all eggs must reach the consumer within a maximum of 21 days of being laid or at least 7 days prior to the declared best before date (28 days maximum from lay) whichever is the sooner.

From effect of 1 January 2004 a code indication of the producers unique distinguishing number must be printed on all Class A eggs eg: 1UK12345. The distinguishing number includes an indication of origin and method of production. The stamp must be legible letters and figures at least 5mm high in food grade ink indelible and resistant to boiling. Small producers selling ungraded eggs are not yet required to stamp eggs, however this will change on 1 July 2005 when all eggs irrespective of flock size will be stamped.

Eggs not reflecting the above criteria are in breach of the egg marketing regulations.

ANNEX 4: LABORATORY METHODOLOGY

Sampling procedure

Wear suitable single-use gloves for handling the eggs, changing the gloves after each batch of six eggs.

Aseptically break open the eggs and separate shell from contents, taking care to avoid contaminating the contents with pieces of shell. If a portion of shell contaminates the contents this sample should be discarded.

For contents: Add the content of six eggs to a sterile stomacher bag (~180mm x 300mm) or a large screw top container placed on a gravimetric diluter (programmed for 50:50 dilution with buffered peptone water (BPW) Add a small amount of BPW and stomach or shake well the eggs for 1 minute. Add further BPW to create a 50:50 dilution. Holding the top of the stomacher bag closed, mix the sample well, seal the bag with a clip and incubate the stomached sample for 18-20h at 37°C ± 1°C.

For shells: Add the shells to a doubled stomacher bag (~180x 3000mm) Place on the gravimetric diluter (programmed for 50:50 dilution with BPW). Add BPW to create a 50:50 dilution, crush the shells down gently. Mix the sample, seal the bag and incubate the stomached sample for 18-20 h at 37°C ± 1°C.

Test procedure

- a) Add 0.1 ml of the incubated BPW to 10 ml Rappaport-Vassiliadis Soya Peptone Broth (RVS) and incubate at 41.5°C ± 1°C for 24 h.
- b) Also, add 1 mL of the same pre-enrichment culture to 10 mL of Muller-Kauffmann tetrathionate novobiocin broth (MKTTn). Place in an incubator at 37°C ± 1°C for 24 hours.

After selective enrichment streak a 10µl loop onto modified Brilliant Green Agar (mBGA) and Xylose Lysine Desoxycholate agars (XLD). Incubate plates for 24 h at 37°C ± 1°C. Colonies on mBGA: red/pink or white opaque colonies with brilliant red/pink zone, on XLD: red with black centre. Re-incubate those plates with no *Salmonella* colonies for a further 22 ± 2 hours, and re-examine for the presence of typical *Salmonella* colonies.

Confirmation

Perform appropriate biochemical tests for *Salmonella* on typical or suspect colonies (3 of each type from each sample) from both mBGA and XLD plates. Isolates showing typical *Salmonella* biochemical reactions should be tested with polyvalent antisera for typical O and H antigens.

Send 3 isolates of each *Salmonella* type on a nutrient agar slope to the Laboratory of Enteric Pathogens, HPA, Colindale for confirmation, serotyping, phage typing, antibiotic susceptibility testing and archiving.

ANNEX B: FOOD STANDARDS AGENCY SURVEY OF *SALMONELLA* CONTAMINATION OF NON-UK PRODUCED SHELL EGGS ON RETAIL SALE: LETTER PROVIDED TO RETAILERS DURING SAMPLING

Insert Council Logo &/or Name

<Date>

Dear

This letter has been given to you by an Environmental Health Officer (EHO) from [insert name of council] Council.

The EHO is authorised by the Council to carry out food sampling work, and has purchased non-UK produced eggs from your premises as a food sample, which is to be used for a food surveillance survey.

The aim of this particular survey is to produce an ongoing estimate of the *Salmonella* contamination in non-UK eggs available to consumers at retail level in the UK. Boxes of 6 or more non-UK eggs are being sampled and tested during a 13-month period.

This survey is being funded by the Food Standards Agency who has commissioned the Health Protection Agency and the LACORS network to carry out the sampling.

Your premises has been visited as one of the retail outlets where people may buy non-UK eggs - the subject of this survey. The eggs purchased from your premises will be taken to [insert name of lab] for testing, and you will be provided

with the results of this testing by a letter from the Food Standards Agency. Please note that the survey is not for enforcement purposes.

The results of the samples taken in this survey will be collated and will form part of a report on the incidence of *Salmonella* in non-UK eggs sold at retail within the UK. This report will be published by the Food Standards Agency. At the end of the survey, in line with Food Standards Agency policy on openness and transparency in relation to food safety and matters of interest to consumers relative to food, individual retailers/producers of the eggs sampled will be published on the Agency's website www.food.gov.uk as part of this report.

Should you have any queries, please contact Dr Chun-Han Chan, Microbiological Safety Division on the following telephone number: 020 7276 8957 or send an E-mail to Chun-Han.Chan@foodstandards.gsi.gov.uk.

Yours sincerely

**ANNEX C: FOOD STANDARDS AGENCY SURVEY OF *SALMONELLA*
CONTAMINATION OF NON-UK PRODUCED SHELL EGGS ON RETAIL
SALE: SAMPLING FORM**

Sampling form



SAMPLER USE ONLY

Retail Premises details

Sampler name Sampler contact no.

Sample ref no. Local authority

Name of shop, number and address
Postcode

Premises type: Market stall Mobile vendor Supermarket
Other please specify

Sample details - Part 1

Purchase date Brand name

Purchase time

Storage temperature (°C)	
Ambient	Chilled
<input type="text"/>	<input type="text"/>

Egg size: Small Medium Large Extra large
Other please specify

Country Packing station code

Production

Packer / producer name (if applicable)

Address of packer/producer
Postcode

Pack size Purchase price Best before date

Comments

The results of the sample will be sent by the co-ordinating centre to the Microbiological Safety Division at the Food Standards Agency. You will be notified by the Food Standards Agency of any *Salmonella* positive results.

If you require information in regard to this survey please contact:

Dr Christine Little
Health Protection Agency Centre for Infections
Environmental & Enteric Diseases Department (CDSC)
61 Colindale Avenue
London NW9 5EQ
Telephone: 0208 327 7931
E-mail: christine.little@hpa.org.uk

LABORATORY USE ONLY

Sample details - Part 2

Condition of eggs

(sample of 6 eggs): Clean

Dirty

Cracked

Other please specify

Comments

Stamp i.e. AB 1 123 / marking on egg shell

Appearance / condition of eggs:

Acceptable

Unacceptable

Date received / checked

LIMS number

Microbiological Results

Shell: Salmonella spp. positive

Salmonella spp. negative

Contents: Salmonella spp. positive

Salmonella spp. negative

Sent for serotyping? Yes No

Date

Comments

ANNEX D: DEFINITION OF A CLASS A EGG, EGG STAMP MARK REQUIREMENTS, AND ARTICLE 33 OF REGULATION (EC) 2295/2003 – TOLERANCE FOR QUALITY DEFECTS

Regulation EC No 2295/2003 specifies the minimum characteristic for quality classes. Only Class A eggs are sold fresh for human consumption. Class A eggs must have a normal shell and cuticle which is clean and undamaged; an air cell not exceeding 6mm in depth; a clear, translucent, gelatinous egg white; a stationary yolk which is visible under candling as a shadow only; be free of foreign substances; be free of extraneous odours; and washing or cleaning by any other means is not permitted. The legal maximum 'best before' date for Class A eggs is 28 days from date of lay. In addition to the 'best before' date, the recommended sell-by date shall not exceed the maximum time limit of 21 days from the date of lay (EC 2003; EC No 853/2004¹), i.e. eggs must be sold to consumers within a maximum of 21 days of being laid or at least 7 days prior to the declared best before date (28 days maximum from lay) whichever is the sooner (MAFF 1996a).

From 2004, Regulation EC No 5/2001 requires all Class A eggs placed on the market within the EU to be marked with a code identifying the production establishment, country of origin and farming system (i.e. organic, free range, barn or cage) (EC 2001). The stamp mark must consist of legible letters and figures at least 5mm high in food grade ink indelible and resistant to boiling. The introduction of stamp marking on egg shells improves consumer information, choice, and assists in traceability of eggs placed on the market for human consumption.

Egg stamp mark codes are in the format X.YY.12345, where:

-
- ¹ Regulation (EC) No 853/2004 of the European Parliament and of the Council laying down specific hygiene rules for food of animal origin. Official J European Union **L226**, 22-82

X is a digit representing the farming method, e.g. 0=Organic, 1=Free Range, 2=Barn, 3=Cage.

YY are letters representing the country of origin, e.g. AT=Austria, BE=Belgium, CY=Cyprus, CZ=Czech Republic, DE=Germany, DK=Denmark, EE=Estonia, ES=Spain, FI=Finland, FR=France, GR=Greece, HU=Hungary, IE=Ireland, IT=Italy, LV=Latvia, LT=Lithuania, LU=Luxembourg, MT=Malta, NL=Netherlands, PL=Poland, PT=Portugal, SE=Sweden, SK=Slovakia, SI=Slovenia, UK=United Kingdom.

12345 are digits representing the unique identification number of the farm.

Eggs not reflecting the above criteria are in breach of the egg marketing regulations

Article 33 of Regulation (EC) 2295/2003 Tolerance for Quality Defects

1. The following tolerances shall be allowed in the checking of batches of Class A eggs and washed eggs:

- (a) at the packing centre, just before dispatch: 5 % of eggs with quality defects,
- (b) at the other marketing stages: 7 % of eggs with quality defects.

However, in the checking of eggs marketed as "extra" no tolerance shall be allowed for the height of the air space either at the time of packing or import.

2. Where the batch checked contains fewer than 180 eggs, the percentages referred to in paragraph 1 shall be doubled.

ANNEX E: EGG PRODUCTION SYSTEMS

Information on egg production systems has been provided by Defra and is published at <http://www.defra.gov.uk/foodrin/poultry/faq/marketing.htm>

Cage

A cage system consists of tiers of cages. The cages have sloping mesh floors so that the eggs roll forward, out of the reach of the birds to await collection. For each cage there must be at least 10cm of feed trough/bird and at least two drinkers/cage or 10cm of drinking trough/bird. Droppings pass through the mesh floors onto boards, belts or into a pit to await removal. A minimum of 550cm squared per bird is required in standard cages, which were installed prior to 2003.

Since 2003 only installation of enriched cages are allowed, with a minimum of 750cm square per bird along with a nest, perching space at 15cm/bird and a scratching area. In each cage feeding troughs must be at least 12cm/bird and at least two nipple drinkers or two cups must be within easy reach of each hen (where nipple drinkers are provided).

Barn

The Barn system has a series of perches and feeders at different levels. The maximum stocking density is 9 birds per square metre and there must be at least 250cm square of litter area/bird. Perches for the birds must be installed to allow 15cm of perch per hen. There must be at least 10cm of feeder/bird and at least one drinker/10 birds. There must be one nest for every 7 birds or 1 square metre of nest space for every 120 birds. Water and feeding troughs are raised so that the food is not scattered.

Free range

In Free-range systems the birds are housed as described in the Barn system above. In addition birds must have continuous daytime access to open runs

which are mainly covered with vegetation and with a maximum stocking density of 2,500 birds per hectare.

In all systems the birds must be inspected at least once a day. At the end of each laying period the respective houses are completely cleared and disinfected.

All EU requirements for the above systems can be found in Council Directive 1999/74/EC (http://europa.eu.int/eur-lex/pri/en/oj/dat/1999/l_203/l_20319990803en00530057.pdf), Commission Regulation 2295/2003/EC (http://europa.eu.int/eur-lex/en/consleg/pdf/2003/en_2003R2295_do_001.pdf), as amended by Commission Regulation 1515/2004/EC (http://europa.eu.int/eur-lex/pri/en/oj/dat/2004/l_278/l_27820040827en00070014.pdf) and Welfare of Farmed Animals (England) (Amendment) Regulations 2002 (<http://www.hmso.gov.uk/si/si2002/20021646.htm>)

Organic

Organic systems are similar to those of free range however there are additional guidelines for feed and veterinary requirements. The pullets should be raised by certified organic production methods from birth. There are derogations available if no organic pullets to be found. These derogations are issued under certain conditions and are strictly controlled. Poultry must have access to out doors whenever the weather permits and for at least a third of their life. Where available organic feed must be used although derogations can be obtained for a restricted amount of non-organic feed where it can be demonstrated that no organic feed can be sourced. These derogations are strictly controlled and issued under certain conditions. Organic livestock systems place great emphasis on positive welfare in disease control by using appropriate breeds and development of health building and disease control methods appropriate to the area of the farm. Welfare plans are a national requirement. Medicinal products are only permitted where it is compulsory legislation or where there is a known risk, or

where it is essential to avoid suffering or distress. The use of veterinary medicines is strictly controlled.

The minimum requirements for organic farming in the UK are set out in the "Compendium of UK Organic Standards" which can be accessed on the Defra website at <http://www.defra.gov.uk/farm/organic>.

ANNEX F: BREAKPOINT CONCENTRATIONS FOR ANTIMICROBIAL DRUGS

Antimicrobial	Abbreviation	Concentration (mg/l)
Ampicillin	A	8
Cefotaxime	Ctx	1
Chloramphenicol	C	8
Ciprofloxacin	Cp	0.125; 1
Furazolidone	Fu	8
Nalidixic Acid	Nx	15
Rifampcin	Rf	64
Spectinomycin	Sp	64
Streptomycin	S	16
Sulphonamides	Su	64
Tetracycline	T	8
Trimethoprim	Tm	2

ANNEX G: NON-UK EGG IMPORT SHARE AND SAMPLE RETURNS

Country	Egg Import Share (%)	Number of Sample Returns		
		Observed	Expected	Weight
Belgium	0.7	13	12	0.92
France	11.1	348	193	0.56
Germany	26.5	45	461	10.25
Netherlands	28.6	129	500	3.87
Poland	1.5	4	26	6.57
Portugal	3.0	25	52	2.07
Republic of Ireland	1.3	23	23	1.02
Spain	21.7	1157	379	0.33

Source: H M Revenue and Customs

Data prepared by Trade statistics, Agricultural Statistics and Analysis Division, Defra

ANNEX H: *SALMONELLA* CONTAMINATION OF SHELL AND CONTENTS SAMPLES FROM NON-UK EGGS (n=1,890)

H.1 Collection and examination of non-UK egg samples in the survey

A total of 1,890 boxes of 6 eggs or more eggs were sampled during the course of the survey. The shells and contents were examined separately (making a total of 3,780 pooled samples examined). One hundred and forty five of the pooled egg samples contained one or more eggs that were found to have visibly cracked shells and one had no country of origin information; thus 146 samples were considered as being of unacceptable condition. Figures and proportions reported for *Salmonella* sero- and phage-types isolated from shells and contents of non-UK eggs within this annex are based on 1,890 pooled samples of both acceptable and unacceptable six eggs rather than individual eggs.

H.2 *Salmonella* serotype, phage-types and antimicrobial susceptibility of isolates from shells and contents

In six samples, two separate salmonellas were detected making a total of 189 distinct isolates recovered from acceptable and unacceptable non-UK egg samples (Table H1). The *Salmonella* isolates comprised eight different serotypes, of which most were *S. Enteritidis* (83.1%; 157/189). Ten different phage types of *S. Enteritidis* were recovered, with PT1 predominating (82.1%; 129/157) (Table H1).

The susceptibility of the 189 *Salmonella* isolates to the panel of 12 antimicrobial drugs is shown in Table H2. The majority of the *Salmonella* isolates were resistant to one or more to the antimicrobial drugs tested (81.5%; n=154), of which most were resistant to nalidixic acid with reduced susceptibility to ciprofloxacin (77.8%; n=147).

Table H.1. *Salmonella* serotypes and phage types isolated from shells and contents of non-UK eggs of both acceptable and unacceptable condition (n=189)*

<i>Salmonella</i> sero / phage types	No. Shells	Positive Pools*	No. Contents	Positive Pools	Total Pools	Positive Pools
<i>S. Enteritidis</i> (all types) [†]	149		8		157	
<i>S. Enteritidis</i> PT1	124		5		129	
<i>S. Enteritidis</i> PT4	1		0		1	
<i>S. Enteritidis</i> PT6a	5		0		5	
<i>S. Enteritidis</i> PT6d	1		0		1	
<i>S. Enteritidis</i> PT7	7		2		9	
<i>S. Enteritidis</i> PT34	2		0		2	
<i>S. Enteritidis</i> PT37	1		0		1	
<i>S. Enteritidis</i> PT53	1		0		1	
<i>S. Enteritidis</i> PT56	5		1		6	
<i>S. Enteritidis</i> RDNC [†]	2		0		2	
<i>S. Braenderup</i>	1		0		1	
<i>S. Infantis</i>	2		0		2	
<i>S. Mbandaka</i>	12		4		16	
<i>S. Panama</i>	2		0		2	
<i>S. Rissen</i>	3		0		3	
<i>S. Unnamed</i> (I O=Rough: g,m:-)	7		0		7	
<i>S. Weltevreden</i>	1		0		1	
Total	177		12		189	

*, 6 samples had 2 separate *Salmonellas*

†, RDNC, reacted with the phage set but did not conform to a recognised phage type

Table H.2. *Salmonella* isolates recovered from shells and contents of non-UK shell eggs (n=189)*

<i>Salmonella</i> sero / phage type	No. isolates	No. isolates fully sensitive	No. isolates resistant	Resistance pattern [‡]
<i>S. Enteritidis</i> (all types)	157			
<i>S. Enteritidis</i> PT1	129	0	129	NxCp _L
<i>S. Enteritidis</i> PT4	1	1	0	
<i>S. Enteritidis</i> PT6a	5	0	5	A (3) NxCp _L (2)
<i>S. Enteritidis</i> PT6d	1	0	1	A
<i>S. Enteritidis</i> PT7	9	0	9	NxCp _L
<i>S. Enteritidis</i> PT34	2	2	0	
<i>S. Enteritidis</i> PT37	1	0	1	NxCp _L
<i>S. Enteritidis</i> PT53	1	1	0	
<i>S. Enteritidis</i> PT56	6	6	0	
<i>S. Enteritidis</i> RDNC [†]	2	2	0	
<i>S. Braenderup</i>	1	0	1	ACp _L
<i>S. Infantis</i>	2	2	0	
<i>S. Mbandaka</i>	16	15	1	SuTTmNxCp _L
<i>S. Panama</i>	2	2	0	
<i>S. Rissen</i>	3	2	1	ASuTTm
<i>S. Unnamed</i> (l O=Rough: g,m:-)	7	2	5	NxCp _L
<i>S. Weltevreden</i>	1	0	1	ASSuSp _L TTm
Total	189	35	154	

*, 6 samples had two separate salmonellas

†, RDNC, reacted with the phage set but did not conform to a recognised phage type

‡, Key to antimicrobials: A, Ampillicin; Cp_L, reduced susceptibility to ciprofloxacin; Nx, Nalidixic acid; S, Streptomycin; Su, Sulphonamides; Sp_L, reduced susceptibility to Spectinomycin; T, Tetracycline; Tm, Trimethoprim

H.4 Country of origin in relation to presence of *Salmonella* spp.

Of the 1,890 pooled eggs sampled, most were produced in Spain (65.1%), France (21.1%) or The Netherlands (7.3%) (Table H3). *Salmonella* spp. were detected from 167 and 3 boxes of eggs produced in Spain and France, respectively. One sample produced in Poland had *Salmonella* spp. present, however the number of eggs produced in Poland sampled (n=5) were comparatively small compared to eggs sampled from other non-UK countries.

Salmonellas were not recovered from eggs produced in Belgium, Germany, Portugal, Republic of Ireland or The Netherlands.

Table H.3. Country of origin of non-UK egg samples and *Salmonella* positive pools

Country of origin	Total No. pools of eggs examined (n=1,890)* (%)		No. pools <i>Salmonella</i> positive (n=171)
Belgium	13	(0.7)	0
France	399	(21.1)	3
Germany	47	(2.5)	0
Poland	5	(0.3)	1
Portugal	28	(1.5)	0
Republic of Ireland	26	(1.4)	0
Spain	1,232	(65.1)	167
The Netherlands	138	(7.3)	0
Not Stated [†]	2	(0.1)	0

*, each sample comprised 6 eggs

A greater number of different *Salmonella* serotypes were recovered from eggs produced in Spain (n=7) compared to those produced in France (n=2) or Poland (n=1) (Table H4). *S. Enteritidis* was recovered from eggs produced in Spain and France. The nine phage types identified amongst *S. Enteritidis* isolates from Spanish eggs included: PT1 (Nx_{Cp_L}; resistant to nalidixic acid with reduced susceptibility to ciprofloxacin), PT6a (A; resistant to ampicillin), PT6a (Nx_{Cp_L}), PT6d (A), PT7 (Nx_{Cp_L}), PT34, PT37 (Nx_{Cp_L}), PT53, PT56 and RDNC. In contrast *S. Enteritidis* PT4 was isolated from eggs produced in France (Table H4).

Table H.4. *Salmonella* isolates recovered from non-UK eggs of both acceptable and unacceptable appearance produced in France, Poland and Spain

Country of Origin	No. Pools positive*	<i>Salmonella</i> sero / phage types and susceptibility to antimicrobials†	No. Isolates
France	3	<i>S. Enteritidis</i> PT4	1
		<i>S. Mbandaka</i> SuTTmNx _{Cp_L}	1
		<i>S. Rissen</i> AsuTTm	1
Poland	1	<i>S. Braenderup</i> AC _{Cp_L}	1
Spain	167	<i>S. Enteritidis</i> (all types):	157
		PT1 (Nx _{Cp_L})	129
		PT6a (A)	3
		PT6a (Nx _{Cp_L})	2
		PT6d (A)	1
		PT7 (Nx _{Cp_L})	9
		PT34	2
		PT37 (Nx _{Cp_L})	1
		PT53	1
		PT56	6
		RDNC†	2
		<i>S. Infantis</i>	2
		<i>S. Mbandaka</i>	15
		<i>S. Panama</i>	2
<i>S. Rissen</i>	2		
<i>S. Unnamed</i> § (Nx _{Cp_L})	5		
<i>S. Unnamed</i> §	2		
<i>S. Weltevreden</i> (ASSuSp _L TTm)	1		

*, 6 samples had two separate salmonellas

†, RDNC, reacted with the phage set but did not conform to a recognised phage type

‡, Key to antimicrobials: A, Ampillicin; Cp_L, reduced susceptibility to ciprofloxacin; Nx, Nalidixic acid; S, Streptomycin; Su, Sulphonamides; Sp_L, reduced susceptibility to Spectinomycin; T, Tetracycline; Tm, Trimethoprim

§, I O=Rough: g,m:-

H5: Results from the samples deemed unacceptable

Of the 1,890 boxes of 6 or more eggs sampled during the course of this survey 146 were deemed unacceptable for testing. The criteria for rejecting eggs from

the main dataset were if the eggs were cracked (n=145) or insufficient data on the eggs or packaging for analysis (n=1).

Salmonella spp. were detected from 14 of the 146 pooled egg samples of unacceptable condition. The *Salmonella* isolates were tested for susceptibility against a panel of 12 antimicrobial drugs. Most of the isolates showed resistance to nalidixic acid with reduced susceptibility to ciprofloxacin (n=10) (Table H5).

Table H5 *Salmonella* isolates recovered from unacceptable non-UK shell eggs (n=16)

<i>Salmonella</i> sero/phage types	No. positive shell pools	No. positive contents pools	Total no. positive pools	Resistance pattern [†]
<i>S. Enteritidis</i>	9	1	10	
<i>S. Enteritidis</i> PT1	8	1	9	Nx _{Cp_L}
<i>S. Enteritidis</i> PT4	1	0	1	
<i>S. Infantis</i>	1	0	1	
<i>S. Mbandaka</i>	1	1	2	
<i>S. Panama</i>	1	0	1	
<i>S. Rissen</i>	1	0	1	
<i>S. Unnamed</i>	1	0	1	Nx _{Cp_L}
Total	14	2	16	

[†]Key to antimicrobials: Cp_L, reduced susceptibility to ciprofloxacin; Nx, nalidixic acid,

The fourteen *Salmonella* contaminated egg samples of unacceptable condition originated from France and Spain. In total there were six different serotypes of which *S. Enteritidis* was the most common (n=9). Of the egg samples contaminated with *S. Enteritidis*, PT1 predominated (n=8). All the *S. Enteritidis* PT1 isolates were obtained from eggs produced in Spain, and the one *S. Enteritidis* PT4 isolate was recovered from an egg sample originating from France (Table H6).

Table H6 *Salmonella* isolates (shell and contents) recovered from non-UK eggs of unacceptable condition produced in France and Spain.

Country of origin	No. pools positive	<i>Salmonella</i> sero/ phage types and susceptibility to antimicrobials†	No. isolates
France	1	S. Enteritidis (all types)	1
		PT4	1
Spain	13	S. Enteritidis (all types)	8
		PT1 (NxCpL)	8
		S. Infantis	1
		S. Mbandaka	1
		S. Panama	1
		S. Rissen	1
		S Unnamed	1

†Key to antimicrobials: Cp_L, reduced susceptibility to ciprofloxacin: Nx, nalidixic acid,