

**ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD**

**SURVEY OF *SALMONELLA* CONTAMINATION OF UK EGGS**

The attached note reports on the ACMSF Surveillance Working Group's recent consideration of a draft protocol for a survey of *Salmonella* contamination of UK-produced shell eggs on retail sale.

**Secretariat  
March 2003**

## **SALMONELLA CONTAMINATION OF UK EGGS : REPORT ON THE WORK OF THE ACMSF SURVEILLANCE WORKING GROUP**

### **Purpose**

1. The purpose of this paper is to report on the recent involvement of the ACMSF Surveillance Working Group in considering a draft protocol for a Food Standards Agency (FSA) survey of *Salmonella* contamination of UK-produced shell eggs on retail sale.
2. The Working Group's membership comprises Professor Humphrey (Chairman), Professor Gasson, Mrs Jefford, Mr Kyriakides and Dr O'Brien.

### **Background**

3. In its Second Report on *Salmonella*, published in 2001, the ACMSF concluded that the fall in human salmonellosis due to infection with *Salmonella* Enteritidis seen from 1998 was probably due to a reduction in the prevalence of *Salmonella*-contaminated eggs. The Committee said that, taking all the available evidence together, including the trends in vaccination in UK laying flocks and *Salmonella* infection in humans, it believed that vaccination had had a significant effect on the prevalence of egg contamination and human infection.
4. The Committee noted that it had received a considerable volume of information from the egg industry on the results of their *Salmonella* testing programmes. This had provided helpful indicators of the efficacy of the Lion Code vaccination programme which, in the absence of results from Government-funded surveillance, the Committee were minded to take as indicative of that sector of the industry.
5. However, the ACMSF recommended immediate Government-funded surveillance to assess whether the overall level of contamination in UK hens' eggs had reduced since the survey carried out in 1995/96, including a comparison between eggs from vaccinated flocks and eggs from flocks where control measures did not include vaccination. The Committee noted that the survey would be enhanced if it were possible to incorporate differentiation between cage-produced eggs and free range production. If this were not possible, then great care should be taken to reflect the weighting of the various production systems in the sampling protocol.

### **Surveillance at egg packing stations**

6. In June 2002, the FSA wrote to egg industry organisations proposing a survey based on the collection of samples at egg packing stations. This, it was felt, would offer the best means of linking the *Salmonella* results to the corresponding on-farm control measures. In the event, the Agency was

unable to secure sufficient industry cooperation to mount such a survey and decided to move instead to retail sampling.

### **Retail survey**

7. In December 2002, the Surveillance Working Group was invited to comment on a draft protocol for a survey of *Salmonella* contamination of UK-produced shell eggs on retail sale. A copy is at Annex A. A copy of the Working Group's comments is at Annex B.

**ANNEX A**



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

**DRAFT PROTOCOL**

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# 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.<sup>1</sup> This work co-incided with the publication of a Department of Health funded survey<sup>2</sup> of the prevalence of *Salmonella* contamination of individual eggs from high street retailers, which indicated a contamination rate of 1 in 650 eggs.
2. A further survey in 1995/6 suggested little improvement, with *Salmonella* being present in 1 in 560 eggs.<sup>3</sup> In view of this, the ACMSF set up a second *Salmonella* in eggs working group.
3. In the mid 1990s vaccination of certain chickens against *S. enteritidis* commenced and it is currently believed that at least 85% of all laying hens in the UK are vaccinated in this manner. The number of laboratory confirmed cases of human salmonellosis has shown a steady decrease since 1998<sup>3</sup>. The working group concluded that this was probably due mainly to the impact of vaccinating chickens against this serotype in line with the earlier introduction of controls on breeding flocks and improved biosecurity. In its 2<sup>nd</sup> *Salmonella* in eggs working group report, the ACMSF recommended that this theory be validated via a new survey. The purpose of the present survey is therefore to assess the current levels of contamination and the effect of measures introduced in recent years (including vaccination).

## Objectives

4. The primary objective of the survey is to:
  - Determine the level of *Salmonella* contamination of UK produced shell eggs on retail sale. This will show whether the level of *Salmonella* contamination has changed since the previous survey in 1995/6 in the light of various interventions, the main one of which is vaccination.

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

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

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

5. It should be noted that the survey is not intended to cover imported eggs or provide information on seasonality. There is a very small chance that imported eggs will be sampled as they are generally not identifiable at retail. However, as far as possible, the aim will be to identify the source of all *Salmonella* positive eggs and, if imported, eliminate them from the final results. It should be noted that a scoping study to investigate levels of contamination in imported eggs is being planned with a view to undertaking a larger scale survey.

### 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 and packing station codes. 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 whether the level of *Salmonella* contamination of UK produced eggs has changed since the previous survey in 1995/96. The number of eggs required to be tested to achieve this objective depends on the degree to which the rate of contamination has changed. For example, to detect a 25% reduction would require 20,000 boxes of six eggs to be tested but to detect a 50% reduction would only require 2,500 boxes. Since the degree to which the rate has changed is unknown, the decision has been taken to start by testing 2,500 boxes, assess the results and take more samples, as necessary until a change is detectable.
9. The basis for the sampling plan is that the samples will be collected in proportion to the market share for each production type<sup>4</sup>, as follows:

|                                  |     |
|----------------------------------|-----|
| • Cage                           | 69% |
| • Free Range (excluding organic) | 23% |
| • Barn                           | 6%  |
| • Organic                        | 2%  |

However, in order to gain enough data to assess whether a follow up study is needed focusing on differences between production types, it will be necessary to over sample the minority production types. The core sample for this survey will therefore be **3,600** boxes, made up of 1,800 boxes of cage eggs and 600 samples each of free range, barn and organic eggs.

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<sup>4</sup> Defra Eggs Statistic Notices

10. The UK core sample of 3,600 will be distributed amongst the countries, according to a weighted percentage based on population size times egg consumption<sup>5</sup>, as follows:

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

The core sample plan is shown in Appendix 2. This has been calculated based on the total market share<sup>6</sup> amongst the retailers taking into account, as far as possible, sales of the individual production types at these outlets and the availability of stores across the UK.

11. Scotland, Wales and Northern Ireland will require over-sampling in order to provide data that can be examined in terms of the different countries. It has been calculated that the sample size should be 778 in each of Scotland, Wales and Northern Ireland. This figure is based on 600 boxes to provide an effective sample size for the individual country, plus an additional 178 samples to even out the distribution of the minority production types across these countries. This would give a total of **5,300** boxes with the following additional samples required in each of the countries.

- Scotland 439 boxes
- Wales 598 boxes
- Northern Ireland 663 boxes

**[NB. This sample plan will be completed once the above countries have made a decision on whether they wish to be over-sampled]**

12. The contractor will be responsible for ensuring that the appropriate number of samples (as outlined in Appendix 2) are collected in accordance with the sampling plan and that sampling is distributed as uniformly as possible throughout the survey period. The contractor should aim to follow the sampling plan as far as possible and if deviations are necessary (e.g. due to a retailer not being available in a particular area) these should be noted in the report to the Agency. 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. If unsure of the production type the eggs should not be sampled.

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<sup>5</sup> Data obtained from Defra's National Food Survey 2000

<sup>6</sup> Sourced for the 7 major retailers from Taylor Nelson Sofres for the 52 weeks ending 10<sup>th</sup> November 2002. Other market share data acquired from the British Egg Industry Council website, Mintel Eggs Market Intelligence report June 2002 and general market knowledge.



13. Retailers should receive payment for the eggs at the time of sampling. The contractors will be responsible for organising this payment.
14. Contractors should provide smaller independent retail outlets 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 is not necessary and the relevant contact at head office will be sent a list of their premises from which samples have been obtained.

## **SAMPLING**

### **Sample collection**

15. It is essential that cross-contamination be 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*.
16. 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 evidence that there is an association between size of egg and prevalence of *Salmonella* there is no need for samplers to select specific sizes.
17. Samples must consist of intact eggs with no evidence of damage or of contamination. 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.
18. 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 note that it is not a legal obligation for retailers to state the production type when selling eggs loose in this way and should therefore endeavour to find out the production type when buying these eggs. Samplers should also make certain that the eggs collected are UK produced.
19. Contractors should set in place a contingency plan to ensure enough samples are collected (e.g. to allow for any eggs broken in transit).
20. Contractors should ensure that samples are kept between 5° - 20°C during transportation.

## **Sample information**

21. All relevant information available from the sample should be entered onto a sampling form. This information should include date and time of purchase, retailer and postcode, size, production type, best before date, price and brand name. The contractor will enter this data onto an Excel 97 spreadsheet.
22. We would recommend that each sample is 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 sent for typing.

## **TESTING**

### Receipt of samples

23. On receipt of the samples laboratories should complete a laboratory record form to record details of date received, sample number, best before date, appearance, packing station number (should start with a 9 for UK produced eggs), pack size, egg size, Lion code or other marking, address of packer/producer if available etc.
24. A digital photograph of high resolution should be taken of the packaging and stored on CD-ROM under the appropriate sample number. If this is not possible the contractor must retain all packaging (or a photocopy/photograph of it).
25. Eggs should be held at 5° -20°C and the laboratory sampling procedure should begin within 24 hours of arrival at the laboratory.

### **Examination**

26. Eggs should be examined before testing to confirm the absence of cracks and any bearing marks (e.g. faecal material, dust, mud, blood and the contents of other eggs) other than natural markings will be considered dirty. If any egg from a box of 6 is discovered to be dirty or cracked the entire box should be discarded and one of the contingency samples used in its place.
27. 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). The contractor should carry out examination in areas dedicated to the examination of survey samples and clearly separated from potentially contaminated materials such as meat or chicken. It is also important that the laboratory control strain should be a rare serotype of *Salmonella*.

### **Salmonella methodology**

28. The methodology for *Salmonella* testing is set out in Appendix 3.

### **Data handling and reporting**

29. At fortnightly intervals the contractor will submit to the Agency a summary report containing details of the samples collected to date.
30. An interim report (electronic and hard copy) containing a summary of the results to date will be submitted to the Agency at 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.
31. 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.
32. 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**

33. In order to ensure a high level of accuracy in data entry, checking and backup the contractor 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. PHLS, FEPAS). This should include the testing of External Quality Assessment samples at regular intervals during the survey.

### **Zoonoses Order 1989**

34. Under the Zoonoses Order 1989, laboratories, which isolate *Salmonella* from foodstuffs, must provide Defra (and DARDNI in Northern Ireland) with a listing of subtype found together with the name of the retailer where the eggs were purchased.

## APPENDICES

### APPENDIX 1: PROPOSED TIMETABLE FOR SURVEY

|                            |   |
|----------------------------|---|
| Mid December 2002          | Revised protocol out to consultation          |
| 22nd January 2003          | Meeting with industry                         |
| Late January 2002          | Protocol finalised                            |
| February 2003              | Staff recruitment and training                |
| 1 <sup>st</sup> March 2003 | Start of the survey, sample collection begins |
| Late May 2003              | End of sampling and testing period            |
| Late June 2003             | Contractors report sent to FSA                |
| Autumn 2003                | FSA publishes brief results of survey         |
| Late 2003                  | Final report published                        |

**APPENDIX 2: SAMPLING PLAN FOR CORE SAMPLE OF 3600 BOXES OF EGGS [NB: THIS IS LIKELY TO CHANGE IN THE FINAL PROTOCOL TO ALLOW FOR OVER-SAMPLING IN SCOTLAND, WALES OR NORTHERN IRELAND]**

| RETAILER     | ENGLAND     |            |            |            | NORTHERN IRELAND |           |            |           | SCOTLAND   |           |            |           | WALES     |           |            |           |
|--------------|-------------|------------|------------|------------|------------------|-----------|------------|-----------|------------|-----------|------------|-----------|-----------|-----------|------------|-----------|
|              | Caged       | Barn       | Free range | Organic    | Caged            | Barn      | Free range | Organic   | Caged      | Barn      | Free range | Organic   | Caged     | Barn      | Free range | Organic   |
| Asda         | 280         | 36         | 39         | 25         | n/a              | n/a       | n/a        | n/a       | 38         | 5         | 3          | 1         | 22        | 3         | 1          | 1         |
| Budgen       | 2           | 3          | 3          | 1          | n/a              | n/a       | n/a        | n/a       | n/a        | n/a       | n/a        | n/a       | n/a       | n/a       | n/a        | n/a       |
| Co-op        | 66          | 2          | 26         | 21         | 6                | 2         | 1          | 1         | 6          | 0         | 3          | 3         | 3         | 0         | 1          | 1         |
| Iceland      | 10          | 5          | 6          | 1          | 1                | 1         | 1          | 1         | 1          | 1         | 0          | 0         | 0         | 0         | 1          | 0         |
| Kwik Save    | 3           | 3          | 3          | 2          | n/a              | n/a       | n/a        | n/a       | 0          | 1         | 0          | 0         | 1         | 0         | 1          | 0         |
| M&S          | n/a         | n/a        | 11         | 8          | n/a              | n/a       | 1          | 1         | n/a        | n/a       | 4          | 2         | n/a       | n/a       | 2          | 1         |
| Morrisons    | 127         | 2          | 20         | 5          | n/a              | n/a       | n/a        | n/a       | 16         | 1         | 3          | 1         | 10        | 0         | 1          | 1         |
| Safeway      | 84          | 82         | 33         | 44         | 4                | 3         | 2          | 2         | 4          | 7         | 2          | 3         | 3         | 4         | 2          | 2         |
| Sainsbury    | 237         | 7          | 72         | 167        | 6                | 1         | 3          | 3         | 20         | 1         | 6          | 12        | 9         | 1         | 3          | 7         |
| Somerfield   | 123         | 2          | 13         | 4          | n/a              | n/a       | n/a        | n/a       | 17         | 1         | 2          | 1         | 9         | 0         | 1          | 0         |
| Spar         | 2           | 2          | 2          | 1          | 1                | 1         | 1          | 0         | 0          | 0         | 0          | 0         | 0         | 0         | 0          | 0         |
| Tesco        | 312         | 164        | 87         | 112        | 17               | 5         | 4          | 3         | 27         | 15        | 5          | 9         | 11        | 5         | 5          | 3         |
| Waitrose     | n/a         | n/a        | 12         | 7          | n/a              | n/a       | 1          | 2         | n/a        | n/a       | 2          | 2         | n/a       | n/a       | 1          | 1         |
| Independent  | 57          | 39         | 37         | 19         | 3                | 2         | 1          | 2         | 5          | 3         | 3          | 2         | 3         | 2         | 2          | 1         |
| Farm shops   | 55          | 34         | 31         | 21         | 4                | 2         | 1          | 1         | 8          | 5         | 5          | 4         | 4         | 2         | 2          | 3         |
| Milkmen      | 38          | 28         | 26         | 17         | 6                | 0         | 1          | 0         | 8          | 6         | 5          | 4         | 2         | 2         | 1          | 1         |
| Butchers     | 27          | 24         | 20         | 10         | 2                | 0         | 1          | 0         | 5          | 4         | 4          | 3         | 4         | 4         | 1          | 1         |
| Markets      | 38          | 27         | 25         | 15         | 2                | 2         | 1          | 2         | 6          | 4         | 4          | 4         | 4         | 4         | 2          | 3         |
| Greengrocers | 11          | 19         | 17         | 7          | 4                | 0         | 0          | 0         | 4          | 1         | 2          | 3         | 2         | 2         | 1          | 2         |
| Other        | 11          | 15         | 11         | 7          | 2                | 0         | 0          | 1         | 4          | 2         | 4          | 3         | 3         | 1         | 2          | 2         |
| <b>TOTAL</b> | <b>1483</b> | <b>494</b> | <b>494</b> | <b>494</b> | <b>58</b>        | <b>19</b> | <b>19</b>  | <b>19</b> | <b>169</b> | <b>57</b> | <b>57</b>  | <b>57</b> | <b>90</b> | <b>30</b> | <b>30</b>  | <b>30</b> |

## APPENDIX 3: 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.

**For contents:** Add a small amount of Buffered Peptone Water (BPW) to the contents and stomach. Add further BPW in order to create a 50:50 dilution and stomach again. Incubate at 37°C for 48 hours.

**For shells:** Crush the shells down and add enough BPW to the broken shells to ensure that they are completely covered, shake gently, and add further BPW in order to create a 50:50 dilution. Incubate at 37°C for 48 hours.

### Test procedure

- a) Add 0.1 ml of the inoculated BPW to 10 ml Rappaport-Vassiliadis Soya Peptone Broth (RVS) and incubate at  $41.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  for 24 h.
- b) Also, add 10 ml of the inoculated BPW to 90 ml Selenite Cystine Broth with added Sodium Biselenite (SCB) (4g/l) and incubate at  $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  for 24 h.

After selective enrichment streak a 10 $\mu$ l loop onto modified Brilliant Green Agar (mBGA) and Xylose Lysine Desoxycholate agars (XLD). Incubate plates for 24 h at  $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ . Colonies on mBGA: red/pink or white opaque colonies with brilliant red/pink zone, on XLD: red with black centre. Plates should not be incubated for longer than 24 h, as this will encourage growth of other flora.

### Confirmation

Perform appropriate biochemical tests for *Salmonella* on typical or suspect colonies (3 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 one isolate of each *Salmonella* type on a nutrient agar slope to a reference laboratory for confirmation, serotyping, phage typing, antibiotic susceptibility testing and archiving.

## Advisory Committee on the Microbiological Safety of Food

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17 January 2003

Dear Sonia

### **FOOD STANDARDS AGENCY SURVEY OF SALMONELLA CONTAMINATION OF UK-PRODUCED SHELL EGGS ON RETAIL SALE**

1. Thank you for your e-mail of 23 December inviting ACMSF comments on the draft protocol for the above survey. I have consulted the Committee's standing Surveillance Working Group. The Group's comments are at Annex 1.
2. In the Group's view, a survey of imported eggs should also be conducted as a matter of urgency. The Group's preference would be for surveys of UK and imported eggs to be carried out concurrently, the better to inform the Food Standards Agency's risk management decisions.
3. I am copying this letter to the Chairman (Tom Humphrey) and members (Mike Gasson, Patricia Jefford, Alec Kyriakides and Sarah O'Brien) of the ACMSF Surveillance Working Group.

Yours sincerely

By e-mail

**COLIN MYLCHREEST**  
Administrative Secretary

**ACMSF SURVEILLANCE WORKING GROUP : COMMENTS ON DRAFT  
PROTOCOL FOR FSA SURVEY OF *SALMONELLA* CONTAMINATION OF  
UK-PRODUCED SHELL EGGS ON RETAIL SALE**

| <i>Protocol</i> |              | <b>Comments</b>   |
|-----------------|--------------|---|
| <b>Para.</b>    | <b>Lines</b> |   |
| 1               | 6-7          | <i>Salmonella enteritidis</i> to read <i>Salmonella</i> Enteritidis   |
| 1               | 11           | Add, at end of final sentence, “on either the shell and/or in the contents”.  |
| 3               | 1            | Replace “chickens” with “flocks of laying hens”.  |
| 3               | 1            | <i>S. enteritidis</i> to read <i>S. Enteritidis</i> .   |
| 3               | 6            | Replace “in line” with “together”.  |
| 3               | 9            | Replace “this theory” with “the implication that the reduction in <i>Salmonella</i> cases was linked to a fall in egg contamination rates”.   |
| 12              | 12           | Add between “If” and “unsure”, “the people taking the samples are”.   |
| 16              |              | The temperature of the eggs on retail sale should be recorded, as should information on whether they were displayed in a temperature controlled environment (eg. shop window, air controlled aisle, etc). |
| 17              | 1-2          | Define “contamination” ? Does it mean faeces, blood, feathers, etc ?  |
| 20              | 1-2          | Data logger should be placed with the samples to monitor compliance with transport temperature requirements.  |
| 25              |              | If the age of the eggs is important, then a maximum time should be stipulated between sampling and submission to the laboratory.  |



| <i>Protocol</i> |              | <b>Comments</b>   |
|-----------------|--------------|---|
| <b>Para.</b>    | <i>Lines</i> |   |
| 27              |              | <ul style="list-style-type: none"> <li>• Need for thorough cleaning between sample batches as <i>Salmonella</i> can spread easily and can persist on surfaces for months.</li> <li>• The laboratory in question should provide the FSA with the necessary assurance that the required numbers of expert staff will be available to do the work and that the protocol will be strictly adhered to.</li> <li>• There should be environmental sampling of the laboratory for <i>Salmonella</i> prior to egg testing, as a further safeguard that any contamination found during egg testing originated from the eggs.</li> </ul> |
| Appendix 2      |              | Most Spar outlets are “independents”. They can purchase through the Spar network but are not required to do so exclusively.   |
| Appendix 3      | 3-4          | “Aseptically break open the eggs....with pieces of shell”. How should this be done? What happens if a piece of shell falls into the egg contents ? Should the sample be discarded ?   |