



**A microbiological survey of pre-packed
ready-to-eat sliced meats at retail in UK
small to medium sized enterprises (SME'S).**

FSA project FS241042

Technical Report

CONTENTS	
ABBREVIATIONS	4
GLOSSARY	5
LIST OF TABLES	6
LIST OF FIGURES	7
LIST OF APPENDICES	8
ACKNOWLEDGEMENTS	9
EXECUTIVE SUMMARY	10
1. INTRODUCTION	
1.1. Background	12
1.2. <i>Listeria</i> spp. and food	12
1.3. RTE sliced meats in the UK	12
1.4. Objectives for the survey of RTE sliced meats in SME	13
2. METHODS	
2.1. Survey design	14
2.2. Sampling	
2.2.1. Location of premises sampled	14
2.2.2. Number of samples collected	14
2.2.3. Sample officer training	16
2.2.4. Sample collection and transportation	16
2.2.5. Sample information collected	17
2.2.6. Sample receipt	17
2.3. Sample analysis	
2.3.1. Reporting of <i>Listeria</i> spp. results	18
2.3.2. Sample preparation	18
2.3.3. Enumeration and detection of <i>Listeria</i> spp.	19
2.4. Microbiological Criteria Regulation (EC) 2073/2005	19
2.5. Internal and External Quality Assurance (IQA/EQA) samples	19
2.5.1. Microbiology	19
2.5.2. Chemistry	
2.5.2.1. Salt (sodium chloride)	20
2.5.2.2. pH	20
2.5.2.3. Water activity (a_w)	20
2.6. Data handling and reporting	21
2.7. Statistical analysis	21
3. Results	
3.1. Approach to statistical analyses	22
3.2. Collection and examination of RTE sliced meats	22
3.3. Temperature of RTE sliced meats in SME at time of purchase	24
3.4. Prevalence of <i>Listeria</i> spp. in RTE sliced meats in SME	24
3.5. Presence of <i>Enterobacteriaceae</i> in RTE sliced meats in SME	26
3.6. Presence of <i>Escherichia coli</i> in RTE sliced meats in SME	26
3.7. pH	27
3.8. Salt content	27
3.9. Water activity	30

3.10.	Shelf life	30
4.	DISCUSSIONS	32
5.	CONCLUSIONS	36
6.	REFERENCES	37
7.	APPENDICIES	
7.1.	FSA requirement document	40
7.2.	Thermal imaging of chillers	41
7.3.	Methodology of analyses	43
8.	FSA Draft protocol	54

ABBREVIATIONS

ACMSF	Advisory Committee on the Microbiological Safety of Food
a_w	Water activity
BPW	Buffered peptone water
°C	Degree Celsius
CFA	Chilled Food Association
cfu	Colony forming units
CI	Confidence interval
Defra	Department for Environment, Food and Rural Affairs
DH	Department of Health
EC	European Commission
EFSA	European Food Safety Authority
EQA	External Quality Assurance
FSA	Food Standards Agency
g	Gram
GLP	Good Laboratory Practice
h	Hour(s)
HPA	Health Protection Agency
IQC	Internal Quality Control
ISO	International Standards Organisation
LIMS	Laboratory Information Management System
mBGA	Modified brilliant green agar
ml	Millilitres
MRD	Maximum recovery diluent
PHLS	Public Health Laboratory Services
QC	Quality Control
RTE	Ready-to-eat
RVS	Rappaport-Vassiliadis soya peptone broth
s	Seconds
SME	Small and medium-sized enterprises
TBX	Tryptone bile X- glucuronide
UKAS	United Kingdom Accreditation Service
XLD	Xylose lysine deoxycholate agar

GLOSSARY

Chi-squared test is a statistical method which is used to test whether two categorical variables are dependent on each other. In this report the Chi-squared test was used to compare variables when prevalence numbers were not large enough to complete a logistic regression.

Logistic regression is a modelling tool which is used to analyse the likelihood of an event (e.g. presence of *Listeria* spp. in sliced meat) in relation to relevant variables (such as pack temperature).

Water activity (a_w) is a dimensionless quantity used to represent the energy status of water in a system. It is defined as the vapour pressure of water above a sample divided by that of pure water at the same temperature; therefore, pure distilled water has a water activity of exactly one. The water activity of a food is a measure of availability of water for the metabolic activity and growth of micro-organisms.

Confidence interval (CI) A confidence interval gives an estimated range of values which is likely to include an unknown population parameter, the estimated range being calculated from a given set of sample data. Confidence intervals are usually calculated so that this percentage is 95%.

LIST OF TABLES

Table	Title	Page
Table 1.	Presence of <i>Listeria</i> spp. in samples of pre-packaged RTE meats.	25
Table 2.	Reported prevalence of <i>Listeria monocytogenes</i> in pre-packed ready to eat meats.	35

LIST OF FIGURES

Figure	Title	Page
Figure 1	Distribution of SME premises visited in the UK, during sampling for RTE meats.	15
Figure 2	Proportion of samples produced in the UK, Ireland (IE), Germany (DE) and Belgium (BE).	23
Figure 3	Distribution of meat types comprising samples of RTE meats in SME.	23
Figure 4	Distribution of temperatures measured on RTE meats sampled in SME during the survey.	24
Figure 5	Distribution of <i>Enterobacteriaceae</i> counts (\log_{10} cfu/g) found in RTE meats (n=1049).	26
Figure 6	Distribution of the pH in RTE meat samples (n=1049).	26
Figure 7	Distribution of salt content in samples as g/100g in 1048 samples.	28
Figure 8	Comparison of salt levels found by laboratory analysis with those declared on the pack label.	29
Figure 9	Distribution of water activity (a_w) in samples (n=1049).	30
Figure 10	Distribution of remaining shelf life of samples on day of purchase.	31
Figure 11	Distribution of temperatures measured on RTE meats sampled in SME during the survey (UK) in comparison with those (FSA) found in 2007 in supermarkets in a previous survey (FSA B18024).	33

LIST OF APPENDICES

Appendix	Title	Page
7.1	FSA requirement document	40
7.2	Thermal imaging of chillers	41
7.3	Methodology of analyses	43

ACKNOWLEDGEMENTS

This FSA project ' Microbiological survey of pre-packed ready-to-eat (RTE) sliced meats at retail in UK small to medium sized enterprises, (FS241042), was carried out by Food Microbiology Branch, the Agri-Food & Biosciences Institute, Belfast, and Hutchison Scientific Ltd, Axbridge, Somerset. The former conducted all analytical work, and collected samples within Northern Ireland, whilst the latter undertook all sampling in GB and shipped samples to Belfast for analysis. Hutchison Scientific Ltd also prepared an internet-accessible database into which all supplementary sample data and test results were entered. Reports based on the entered information were accessible to all relevant personnel, including FSA staff, for the duration of the surveillance.

EXECUTIVE SUMMARY

The FSA's Foodborne Disease Strategy (FDS) for 2010-2015 proposes a pathogen-specific approach to reducing foodborne disease rates in the UK and *L. monocytogenes* has been identified as one of the priorities for action. This is because infections with this organism (listeriosis) are associated with a high rate of mortality. It is therefore important that sources of exposure to this organism are pinpointed and factors contributing to infections identified. The types of food in which *Listeria* can be found are diverse and there are several key foods that have been implicated in the transmission of listeriosis, including cooked sliced meats.

A recent HPA study of human listeriosis in England (2001-2007) found that the study cases (particularly the elderly who are a high risk group) were more likely to purchase foods from smaller convenience stores than the general population and so this survey was designed to focus on SME retailers. The primary objective was to determine the prevalence and levels of bacterial contamination in ready-to-eat (RTE) cooked and cured sliced meats at retail sale in small to medium sized enterprises (SME's). A previous FSA survey of retail cold sliced meats with particular reference to *L. monocytogenes* was based on market share (B18024) and therefore the majority of samples were collected from the large retailers. In addition this survey also covered fermented and cured meat products that were excluded from the previous FSA survey.

Overall, 1,049 samples of pre-packed ready to eat sliced meats purchased in SME's in the UK were examined to detect and/or enumerate *Listeria monocytogenes* and other *Listeria* spp. Samples were also examined to determine numbers of the hygiene indicator organisms *Escherichia coli* and *Enterobacteriaceae*, as well as for a range of physical characteristics; salt content, water activity and pH. The temperature of the surface of packs of meat in the SMEs at the time of sampling was measured and recorded, as were a range of other details including price, 'use-by' date and storage instructions.

Overall, *Listeria monocytogenes* was detected in 3.8% of samples and *Listeria* spp. were detected in 7.0% of samples. *Enterobacteriaceae* were enumerated from 36.2% of samples and the mean count (\log_{10} cfu/g) was 2.96 ± 1.47 . *E. coli* were enumerated from five samples (0.48%). The mean temperature of the samples was 6.80°C, with 71.3% of samples above the industry guideline of 5°C and 32.7% being stored above 8°C. The mean pH of the samples was 6.09, and the mean a_w was 0.98. The mean remaining shelf life was 13.8 days, and 75.1% of samples had 14 days or less of shelf life remaining.

Samples came from a diverse range of processors and chemical analyses indicated conditions appropriate for the growth of *Listeria* species. *Listeria* were isolated from the products of 27% of the producers sampled. Product from 23 processors showed a mean *Listeria* prevalence of 25%, indicating significant hygienic shortcomings in the production processes. Therefore a minority of processors appear to be manufacturing pre-packed ready to eat sliced meats of unacceptable quality, based on Guidelines issued by the Health Protection Agency in 2009.

The survey found that most of the meat products were stored at temperatures higher than the recommended industry guidelines, and this, combined with the relatively high

prevalence of *Listeria monocytogenes*, in comparison with the previous FSA survey, could put consumers at risk. There is a need to inform and train retail SME staff about appropriate maintenance and control of refrigerated display cabinets. The manufacturers producing unacceptable RTE meats will require investigation with appropriate corrective actions being taken.

INTRODUCTION

1.1 Background

Non-invasive infection with *L. monocytogenes* can lead to the development of febrile gastroenteritis within the generally immunocompetent human population and the symptoms of this illness are comparatively mild and therefore are not likely to be reported to clinicians (Miettinen et al, 1999). However, particularly in immune-compromised individuals e.g. the elderly and pregnant women, this organism may establish a considerably more severe invasive infection, listeriosis (Lyytikäinen et al, 2006). In this infection, the pathogen can spread through the blood circulatory system and /or the central nervous system with severe consequences including bacteraemia, septicaemia or meningitis. Vulnerable populations may suffer mortality rates of 20-30% (Farber, 2000; Gillespie 2010). Listeriosis in pregnant women can result in spontaneous abortion, stillbirth or the delivery of a severely ill baby due to the infection, although the mother may have much less severe symptoms.

Due to the severity and sequelae of listeriosis, and its association with the consumption of contaminated foods, a reduction of the number of cases of food related listeriosis within the UK has been specifically identified as a priority within the FSA's Foodborne Disease Strategy (FDS) for 2010-2015. It is therefore important to identify the sources of human exposure to *Listeria monocytogenes* in food and the factors contributing to the incidence and severity of food related listeriosis.

Listeria spp. can be recovered from a wide range of foods. These organisms are killed by normal cooking processes, which mean that they are less likely to pose risks within freshly cooked foods. However, contaminated ready to eat (RTE) foods are considered to be significant vehicles for human infection (EFSA, 2007; Gillespie et al. 2004). In particular retail cooked sliced meats have been reported to be frequently contaminated by *L. monocytogenes* (Little et al. 2007).

1.2 *Listeria* spp. and food

L. monocytogenes is ubiquitous on vegetation and in soils and surface waters (Strawn et al 2013) and consequently is routinely isolated from foods such as fresh produce and cold smoked fish, which are not cooked prior to consumption (FAO/WHO, 2004). The opinion of the FAO/WHO (2004) is that it is likely that most consumers routinely ingest small numbers of *L. monocytogenes*, but in raw foods the normal microflora present will compete with *L. monocytogenes* and prevent significant growth. As noted above, retail foodstuffs such as RTE meats are amongst the most frequently contaminated, but the cooking process which most meats undergo will kill a significant proportion of the bacterial population, minimising the competitive flora and allowing *L. monocytogenes* to grow to potentially hazardous numbers.

1.3 RTE sliced meats in the UK

The pre-packed chilled meats sector has existed for some 40 years in industrialised countries. The vast majority of chilled pre-packed meat products utilise modified atmosphere packaging (MAP) or vacuum packing (VP). In the UK, 70% of pre-packed

sliced cooked meats are packed in MAP and 30% under VP (BRC, personal communication; Kaarin Goodburn 20/02/2013).

Within the EU, pre packed sliced cooked meats must be prepared and presented in line with specific food safety/hygiene (178/2002, 853/2004) and labelling legislation (2000/13 until 12/12/14 and subsequently 1169/2011). The specific details of production standards and requirements, and the means for their achievement are developed by processors, bearing in mind the membership requirements of trade associations (e.g. BMPA, CFA) and the conditions of supply defined by large retailers. Given the technical expertise needed to comply with retailer supply standards, and concomitant auditing (including third party), such onerous requirements tend to select for larger, well-resourced businesses.

However, manufacturers who are not members of such trade associations, or do not supply customers with defined requirements, will only be subject to periodic Local Authority checks to ensure the defined minimum legal requirements are met.

1.4 Objectives for the survey of RTE sliced meats in SME

The primary objective of this survey was to determine the prevalence and levels of contamination in RTE cooked and cured sliced meats at retail sale in small to medium sized enterprises (SME's) in the UK. That focus differentiates this study from a previous FSA-funded survey of *L. monocytogenes* in retail cold sliced meats (B18024) which drew most of its samples from larger retailers. A recent HPA (now PHE) study of human listeriosis in England (2001-2007) found that the elderly (a high risk group in relation to listeriosis) were more likely to purchase foods from smaller convenience stores than the general population (Gillespie et al. 2010). This survey also includes fermented, and cured, meat products that were not included in B18024.

The current survey aimed to determine the prevalence of contamination in a range of RTE sliced meats, and to identify potential risk factors associated with RTE sliced meats on sale in SMEs. The survey did not aim to gain specific data on seasonality patterns of *L. monocytogenes* contamination in the sampled products.

Samples were classified in terms of the results of microbiological analyses as specified in 'Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods Placed on the Market' (HPA, 2009).

2 METHODS

2.1 Survey design

The main objective of this study was to obtain data from SMEs for comparison with the prevalence of *Listeria monocytogenes* on RTE sliced meats in supermarkets, as reported in FSA Project B18024. During that study samples were purchased from premises whose address, including their postcode, was recorded. Analysis of these postcodes revealed 1028 unique postcodes, but 45 of these were invalid when checked against the Royal Mail Postcode Address File (PAF) Database. Thus 983 useable postcodes formed the basis of the sampling programme. When these 983 postcodes were plotted on a map of the UK it became clear that there were regional clusters of sampling sites, and these were used as the basis for sampling in this study. This ensured that there was a broad comparability of the areas sampled in the previous FSA study (B18024) and this study (FS241042). In the current study, FS241042, a postcode within each B18024 cluster was used as the starting point in the identification of a sample set.

This survey was designed to recover and analyse at least 1046 samples, to support statistical analysis to determine differences of $\pm 1\%$ (with 95% confidence) between the prevalence of *Listeria monocytogenes* as determined in this study, and 2.8% prevalence of *Listeria monocytogenes* reported in Project B18024 (FSA, 2011b). Data from the enumeration of *Listeria*, *E. coli* and *Enterobacteriaceae* would further inform on potential risks by allowing samples to be rated as satisfactory / borderline / unsatisfactory according to HPA guidelines (HPA, 2009).

2.2 Sampling

2.2.1 Location of premises sampled

The location of premises to be sampled was based on the geographical distribution of premises sampled during FSA study B18024, as noted above. As SME premises were specifically to be sampled in this study sampling officers were provided with a postcode within a sampling cluster defined by the postcodes of premises sampled in B18024. Samplers were instructed to drive to the target area and purchase samples in appropriate premises within the vicinity. The sampling locations are shown in Fig. 1.

2.2.2 Number of samples collected

Two hundred samples were to be purchased in both Scotland and Northern Ireland and 646 in England and Wales, to obtain the required total of 1,046.

Figure 1. Distribution of SME premises visited in the UK, during sampling for pre-packaged RTE meats.



2.2.3 Sampling officer training

Sampling officers in Northern Ireland received agreed standard training and received standard sampling equipment at AFBI (Belfast) from the project leader, Dr R. Madden, and his lab manager, Mrs Lynn Moran.

Sampling officers in GB received agreed standard training and received standard sampling equipment at Hutchison Scientific Ltd. company headquarters (Wolverhampton) from Dr Madden to ensure a consistent approach to sampling by all of the sample collection staff. Training included the aims and objectives for the survey, a review of the operation of equipment such as infra-red thermometers, practical familiarisation with suitable RTE sliced meat products, interpretation and completion of the standardised sample collection forms, and the procedures to be used in the event of sampling problems.

Prior to their issue to sampling officers the thermometers were checked for accuracy using samples held in incubators whose temperatures were calibrated against reference thermometers under ISO17025:2005. The manufacturers, Fluke, state that accuracy is $\pm 1^{\circ}\text{C}$ between 0°C and 65°C , when the thermometer is used at an ambient temperature of $23^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The accuracy of the thermometers was confirmed at the end of the sampling period.

2.2.4 Sample collection and transportation

Sampling officers were provided with a sampling schedule and a list of target postcodes around which they were to identify appropriate SME retail outlets, and collect samples. Sampling officers provided a letter, explaining the survey rationale and processes, to staff at each SME retailer when samples were purchased. A maximum of four random samples (target weight 200g, minimum weight 150g, with at least 2 days remaining shelf-life, and intact and undamaged packaging) were selected from the chilled cabinet(s) in each retail SME site to meet the requirements of laboratory analysis. Where multiple packs were required to meet the above target sample weight multiple packs with identical brand, product, batch identifier and use-by date were collected. At the point of sampling, the surface temperatures of the RTE sliced meats were measured using a non-contact thermometer (Fluke Foodpro infra-red thermometer, Fluke UK Ltd, Norwich, UK) and the result recorded. To avoid cross-contamination all equipment was decontaminated between sampling events, and all samples were stored and transported in resealable sterile bags. The sample packs were placed in a sanitised insulated box (Biotherm 45, DGP Intelsius Ltd, York, UK), with single use cool packs and two temperature recording devices (Tinytag, Gemini Data Loggers (UK) Ltd, Chichester, UK). Product temperature was monitored

and had to be maintained at 0-8°C during transit to the AFBI laboratory for samples to be acceptable. Most samples were delivered to the laboratory within 24hrs. On 3 sampling occasions, chilled transportation times exceeded this target, but no transit times exceeded 48hours. All samples were analysed within their indicated use-by date and within 2 hours of receipt at the AFBI laboratory.

2.2.5 Sample information collected

Data relating to each purchased sample was subsequently added to an internet accessible project database established and maintained by Hutchison Scientific Ltd. This included:

- Sampler name
- Sample reference number
- Retail premises: name address and postcode.
- Premises type: Small convenience, large convenience, garage forecourt, delicatessen, butcher, farm shop, market stall, mobile shop.
- Sample purchase date and time.
- Brand and product description
- Sample temperature
- Number of packs comprising sample.
- Identification number.
- Country of production
- Comments on package type and condition
- Purchase price
- Use-by date
- Display until date

2.2.6 Sample receipt

When sample boxes were received at AFBI chilled sample pack integrity, weight and remaining shelf life were confirmed. Each valid sample was given a unique sample tracking identification number and photographed (front and rear of the packs). Resultant images were transferred to a secure data storage facility. To reduce the possibility of cross-contamination with other foodstuffs undergoing analysis, samples were opened and prepared in sterile airflow cabinets in a laboratory separate from the containment level 2 Laboratory where microbiological analyses took place. Further information on the samples obtained from pack labels, such as the EU processor code, was entered onto the HSL database after sample preparation had been completed. Data was exported from the HSL data base as an Excel spreadsheet at the end of each calendar month, and submitted along with the RTE pack photographs to the FSA for further validation and cross-checking.

2.3 Sample analysis

Microbiological analysis

Microbiological analyses were undertaken in an ISO 17025:2005 accredited laboratory (UKAS 1279). Samples were analysed for the presence of *Enterobacteriaceae*, *Escherichia coli*, *Listeria* spp. and *Listeria monocytogenes*, using appropriate ISO methodologies.

Chemical analysis

Chemical analyses were undertaken in an ISO 17025:205 accredited laboratory (UKAS 1887). pH values, water activities (aw) and salt concentrations in samples were determined using appropriate ISO methods.

2.3.1 Reporting of *Listeria* spp. results

Samples were tested for *Listeria* spp. using detection and enumeration methods based on BS EN ISO 11290-1:1997 and BS EN ISO 11290-2:1998 respectively. The theoretical limit of detection was 1 viable *Listeria* bacterium in 25g of sample for the detection method. The enumeration method for *Listeria* spp. was used to provide an indication of the level of the contamination, and involved direct plating of 0.1ml of the initial 1 in 10 suspension onto a solid selective medium. The theoretical limit of detection was 50 viable *Listeria* bacteria per gram. Should *Listeria* be detected in samples following enrichment, but at levels below the limit of detection of the enumeration protocol then such results were reported as *Listeria* spp. present with a viable count of <10 cfu/g.

2.3.2 Sample preparation

The method of sample preparation was based on the draft protocol provided by the FSA. Methods used and the local reference number were: detection of *Listeria* spp. (including *L. monocytogenes*), NF 13 (based on BS EN ISO 11290-1:1997) and enumeration of *Enterobacteriaceae* NF23 (based on BS EN ISO 21528-2:2004). Enumeration of *Listeria* spp. (including *L. monocytogenes*) as described in BS EN ISO 11290-2:1998, and *E. coli* as described in BS EN ISO 16649-2:2001. In summary microbiological sample suspensions were prepared for enumeration tests (*Listeria* spp., *E. coli* and *Enterobacteriaceae* counts), by weighing 10g of sample into a sterile stomacher bag using aseptic technique. Maximum recovery diluent (MRD) at ambient temperature was added to give a 1 in 10 suspension. The sample was homogenised for 2 minutes using a stomacher; and the homogenised sample was used for each of the enumeration methods. Where the concentration of bacteria was anticipated to require dilution (e.g. *Enterobacteriaceae*), MRD was used to prepare serial tenfold

dilutions from the initial suspension before plating onto agar. For the detection of *Listeria*, 25g of sample was diluted in Fraser broth base and used to prepare a 1 in 10 dilution series, as detailed in the individual methods.

2.3.3 Enumeration and Detection of *Listeria* spp.

Detection methodologies were based on ISO procedures as noted above. For the enumeration of *Listeria* spp. 25g of sample was added to 225ml Fraser broth base (without selective supplements), blended for 2 minutes in a Colworth 400, then allowed to stand for 1 h. Samples were then plated (0.1ml) onto ALOA and PALCAM agar, and incubated aerobically (37°C, 24 to 48 h). Plates with less than 150 typical colonies were counted. Five presumptive colonies from each duplicate plate were then confirmed, using standard ISO phenotypic tests, and the final count obtained by multiplying the presumptive count by the percentage of confirmed *Listeria* colonies.

For the detection of *Listeria* spp. half strength supplements (SR0166) were added to Fraser broth base (CM0895), prepared as above, and incubated (30°C, 24 h), then 0.1ml added to 10ml Fraser broth with full strength supplements (SR0156), which was then incubated (37°C, 48 h). Plates of *Listeria*, Ottavani and Agosti agar, (ALOA CM1084+SR0226) and polymyxin acriflavine lithium chloride ceftazidime aesculin mannitol agar, (PALCAM CM0877+SR0150) were streaked from the broths after incubation, and then incubated (37°C, 48 h), with examination after 24h and 48h. Where plates yielded presumptive *Listeria* five colonies were purified and confirmed using the standard ISO phenotypic tests.

2.4 Microbiological Criteria Regulation (EC) 2073/2005

As agreed prior to the commencement of the study, the FSA was informed when any samples failed the Microbiological Criteria Regulation (EC) 2073/2005 for *L. monocytogenes* in RTE foods (i.e. greater than 100cfu/g).

2.5 Internal and External Quality Assurance (IQA/EQA) samples.

2.5.1 Microbiology

All samples were subjected to routine Internal Quality Control (IQC) and assurance programmes operating within AFBI. IQC procedures involved

spiking a sub sample with a natural bacterial food product derived flora including, *L. innocua*, *L. monocytogenes*, *E. coli*, and *Enterobacteriaceae*.

IQC results obtained for the RTE food samples examined in this study demonstrated satisfactory levels of repeatability among staff and accuracy of analysis between the spiked food matrix and the blank control samples. In the event of out-of-range results, these were repeated by the staff concerned. Food Microbiology branch is accredited by the United Kingdom Accreditation Service (UKAS), accreditation number 1279, to undertake microbiological tests on food for *Listeria* and the other microorganisms studied. Microbiology External Quality Control (EQC) used the QM (Quality in Microbiology) scheme with EQA samples analysed on a quarterly basis. The scheme provided simulated food samples on a variety of matrices for proficiency testing to challenge laboratory procedures. These results were all acceptable for all staff, and a statistical breakdown of individual performance was supplied as part of the report from QM.

2.5.2 Chemistry

2.5.2.1 Salt (sodium chloride)

All samples were tested in accordance with the normal protocols and methods, based on BS 4401-16: 1996 by laboratory staff deemed as competent to conduct the analysis. All standards fell within acceptable limits.

A number of repeat analyses were carried out during the course of the project when a suspect result was obtained. This was normally due to certain cured meat products having an exceptionally high salt content.

2.5.2.2 pH

pH was measured by an in-house calibrated instrument, verified by standardised buffers. Calibration with buffers of pH 7.0 and 4.0 was performed weekly. All samples were tested in accordance with the normal protocols and methods, based on BS 4401 part 9:1975 and BS 770 part 5:1076, by laboratory staff deemed as competent to conduct the analysis. All standards fell within acceptable limits.

2.5.2.3 Water activity (a_w)

Water activity (a_w) was measured using an externally calibrated instrument with routine checks using control samples to ensure validity of calibration. All samples were tested in accordance with the normal protocols and methods, based on ISO 21807:2004, by laboratory staff deemed as competent to conduct the analysis. Samples were held at the measurement temperature prior to analyses to minimise equilibration times. All standards fell within acceptable limits.

2.6 Data handling and reporting

On completion of microbiological and chemical analytical testing, data were entered into the normal lab books and the database. The database was backed up on a weekly basis and the backup stored in a location geographically separate from the web server. All data were checked and authorised by a suitably trained and experienced member of staff before release. Exception reports (presence of *L. monocytogenes*) were raised by AFBI immediately following confirmation of the presence of this pathogen at unsatisfactory levels, as per HPA guidelines. These exception reports were submitted to the Project Manager and communicated to the FSA Project Officer. The Agency was also notified of any trends towards unacceptable results to allow appropriate action to be taken. Results and associated sample photographs and sample data were submitted to the Agency on a regular monthly basis to inform on progress. The data were exported as a single spreadsheet, which was independently cross-checked by Agency staff for accuracy. Agency staff had access to the database allowing random spot checks of sample datasets. Samples found with an insufficient shelf-life, damaged packaging, inadequate photographic records, late delivery to the laboratory, out of specification transit temperature, or which were otherwise unsatisfactory were excluded from testing and/or labelled as unacceptable on the results data spreadsheet.

2.7 Statistical analysis

AFBI has a dedicated team of statisticians who comprise Biometrics Branch, and a member of their staff was designated to work on all aspects this study. On completion of the project and verification of the data on the database the data was exported to an Excel spreadsheet which was supplied to the biometrician. Regression analysis was used to fit the number of *Enterobacteriaceae* (\log_{10} transformed), *Listeria* presence/absence and *L. monocytogenes* presence/absence against each of a number of explanatory variables in turn. For the case of the *Enterobacteriaceae* a normal distribution was assumed and for the other two response variables a binomial distribution with logit link function was used. For each model fitted predictions were formed (with standard errors) for all levels of categorical explanatory variables and for specific values of continuous variables. In addition, within each model, pair wise differences between each predicted value was assessed using Fisher's Least Significant Difference Test, if the overall relationship between response and explanatory variable was found to be significant ($P < 0.05$). In addition the difference between laboratory determined salt levels and pack label salt was fitted against pack salt using linear regression as for number of *Enterobacteriaceae* above.

3 RESULTS

3.1 Approach to statistical analyses

The primary objective of this UK wide survey was to determine the prevalence of levels of contamination in pre-packed RTE sliced meats at sale in small to medium sized enterprises (SME's). Cooked, cold sliced meats e.g. ham, beef, turkey and chicken; sliced cured meats e.g. Parma ham; and sliced fermented meats e.g. salami and chorizo sausage sold at retail premises classed as SME were sampled. Larger retailers were excluded from this survey as they had been heavily sampled in previous FSA surveys, such as B18024, and the EU harmonised survey of *Listeria* in RTE foods.

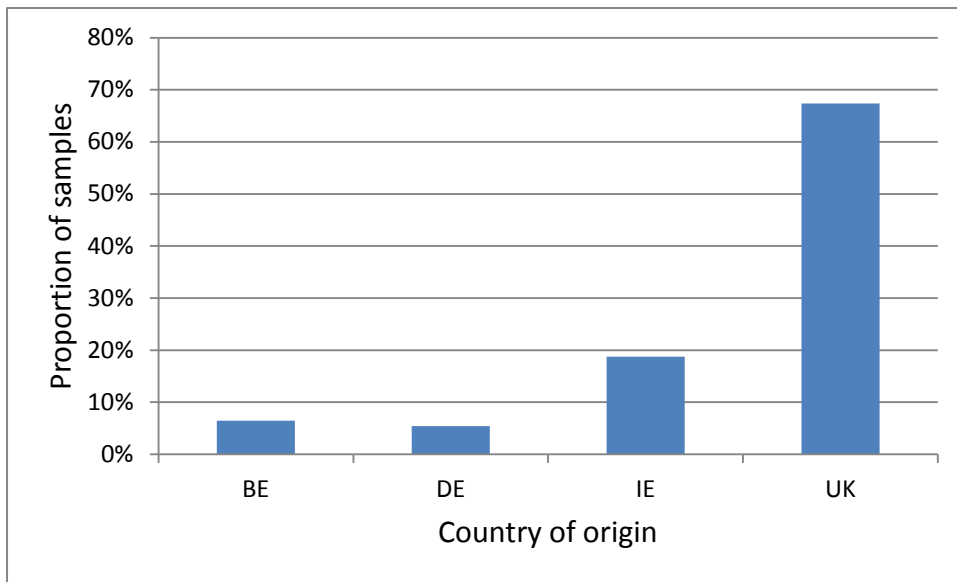
The final sample size was 1,049 RTE meats, excluding invalid samples. One sample had a sell-by date of February 31, 2013 and this was amended to February 28, 2013, to allow statistical analysis to proceed. One sample was not analysed for the presence of salt. The complete results dataset comprised 1048 test samples, and the statistically defined minimal number of samples was 1046 to meet the FSA requirements.

3.2 Collection and examination of RTE sliced meats

Minor errors were made during sampling, e.g. purchase of raw ham, failure to ensure all packs constituting a sample were identical, and purchase of two packs of different product sold as a single item. Regular feedback to sampling officers helped increase awareness of the types of errors made. Overall sampling efficiency was very good, and the project has left a legacy of a pool of trained sampling officers able to undertake UK wide consumer surveys.

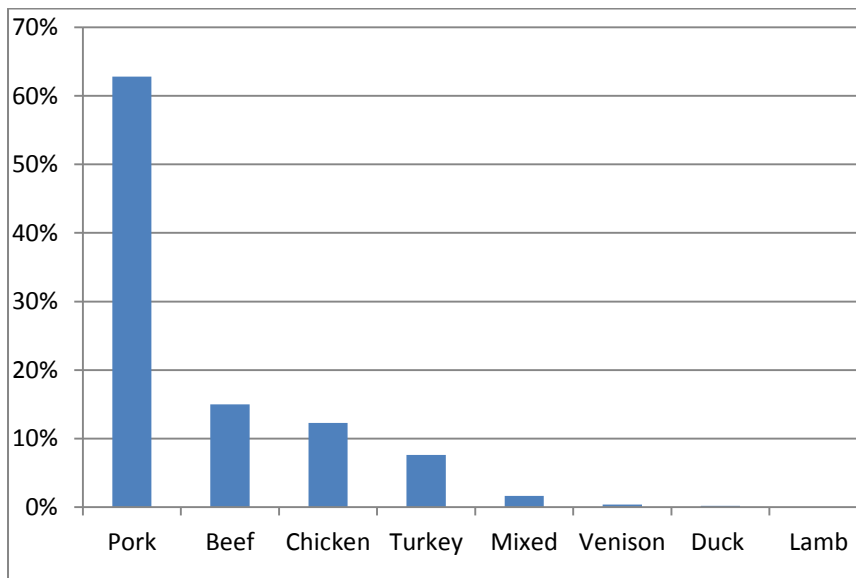
The survey samples were notable for their diversity of processors, with a total of 96 unique EC licence codes being logged. Twenty samples (1.9%) had no producer code. Less than 10 samples were obtained from each of 73 producers, representing 29.6% of all samples, i.e. 75% of producers accounted for less than 30% of samples. Four countries produced 98.1% of samples, Fig 2.

Figure 2. Proportion of pre-packed RTE meat samples produced in the UK, Ireland (IE), Germany (DE) and Belgium (BE).



In terms of the species of meat collected, pork and beef predominated, providing 77.8% of samples, Figure 3.

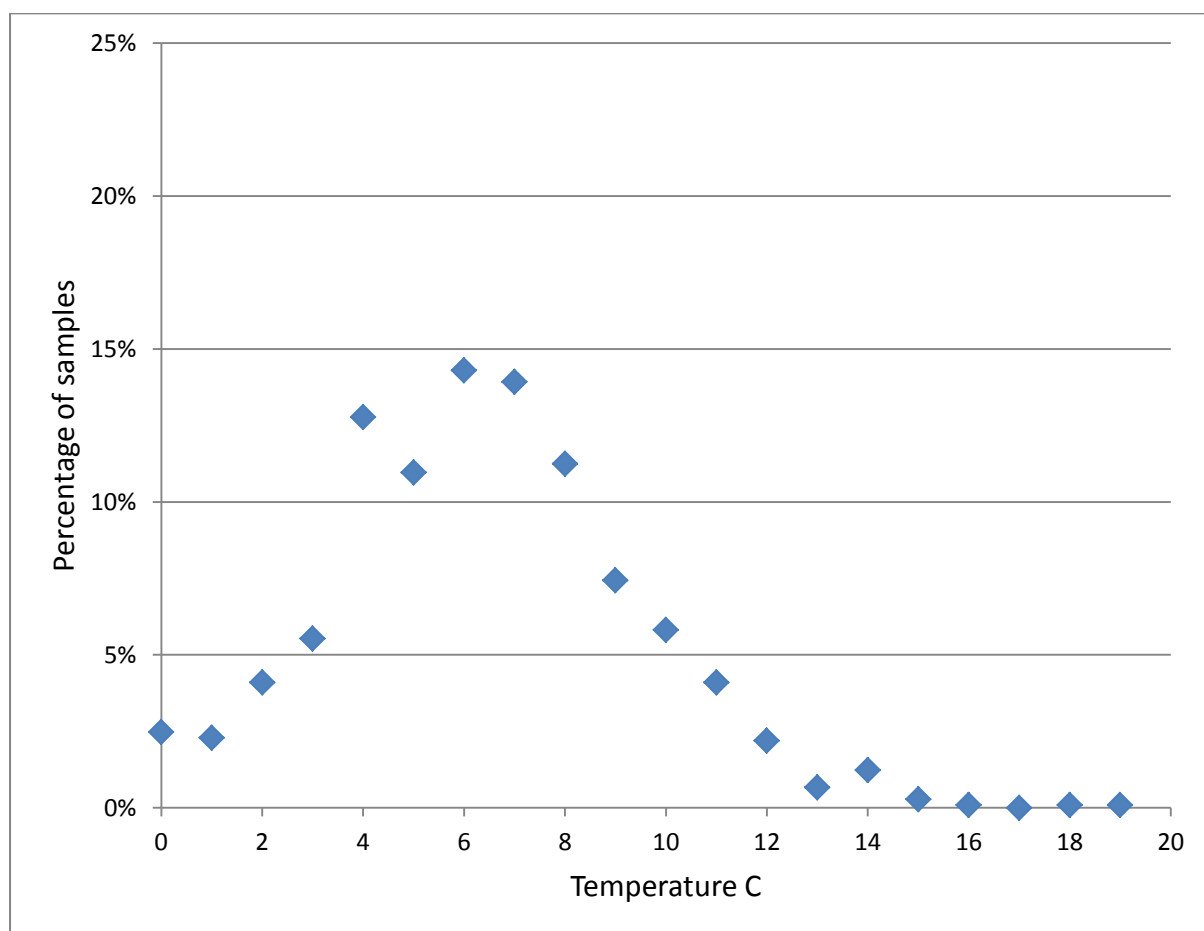
Figure 3. Distribution of meat types comprising samples of RTE meats chosen at random in SME.



3.3 Temperature of RTE sliced meats in SME at time of purchase

The mean temperature of the samples was $6.80^{\circ}\text{C} \pm 3.01$, with 71.3% of samples $\geq 5^{\circ}\text{C}$ and 32.7% $\geq 8^{\circ}\text{C}$, Figure 4.

Figure 4. Distribution of temperatures measured on RTE meats sampled in SME during this survey.



3.4 Prevalence of *Listeria* spp. in RTE sliced meats in SME

Overall, *Listeria* spp. were detected in 7.0% of packs (n=73) (95% confidence interval 5.4% to 8.5%), Table 1, and enumerated in 0.9% of packs (n=9) at a level of 100 cfu/g in eight packs. The remaining pack yielded 200 cfu/g.

Listeria monocytogenes was present in 3.8% of samples (n=40) (95% confidence interval 2.6% to 5.0) and enumerated in 0.4% (n=4).

L. monocytogenes was found at a level of 100 cfu/g, in all four packs.

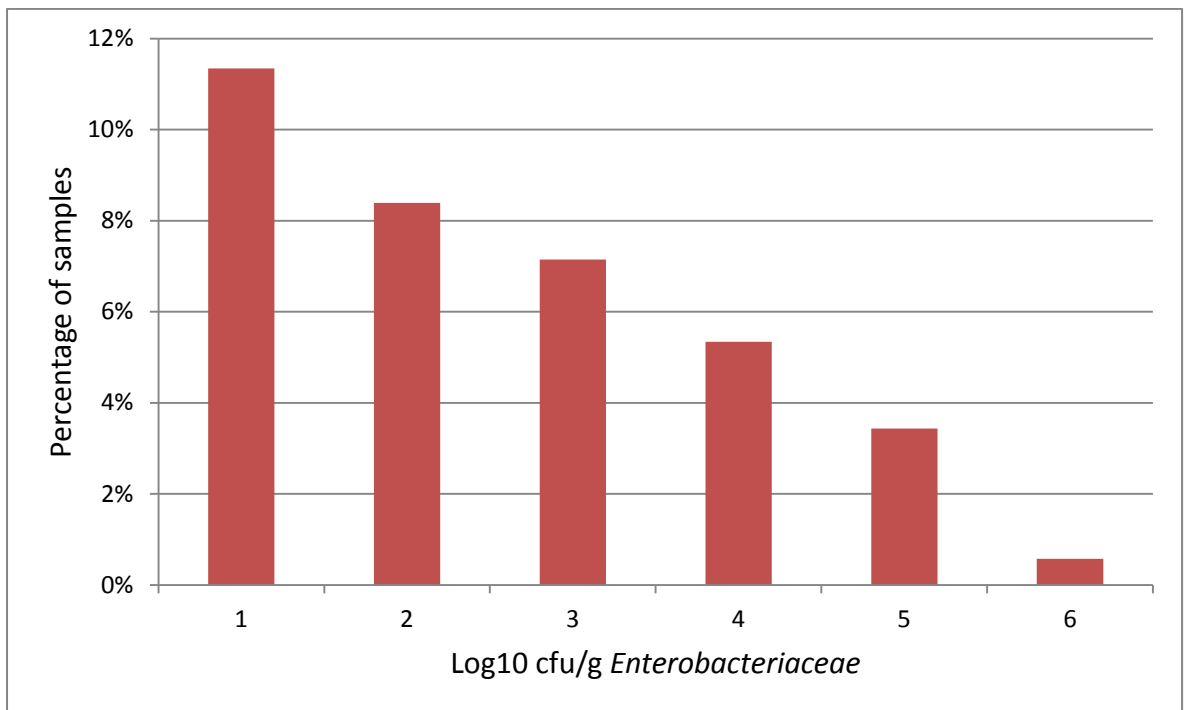
Table 1. Presence of *Listeria* spp. in samples of pre-packaged RTE meats. Samples from 26 processors, and some with no designated EC code, yielded positive samples. Six processors were responsible for 15.8% of all samples tested, but 60.3% of *Listeria* positive samples.

EC Processor Number	Number of samples	Samples positive for <i>Listeria</i> spp (n)	Percentage of samples positive for <i>Listeria</i> spp.
EC UK PC028	22	13	59
EC UK BZ035	68	11	16
EC UK KM080	14	6	43
EC UK FE012	24	5	21
EC UK ZX018	31	5	16
EC UK FP017	7	4	57
EC IE501	177	4	2
GB DR033	2	2	100
GB UK DO013	2	2	100
EC UK MM013	8	2	25
None	20	2	10
EG BE B148	60	2	3
EC UK MQ/C07	1	1	100
EC UK TQ002	1	1	100
EC UK9520	1	1	100
CE ES 10.05655/GE	2	1	50
EC UK LY007	2	1	50
EC UK WZ011	2	1	50
EC UK FP022	3	1	33
EC UK PA011	3	1	33
EF DK885	3	1	33
EG DE NW EUZ230	3	1	33
EC IE575	4	1	25
EC UK WF004	5	1	20
EC UK TC023	12	1	8
EC UK SM025	22	1	5
EC UK TC015	29	1	3

3.5 Presence of *Enterobacteriaceae* in RTE sliced meats in SME

Enterobacteriaceae were enumerated from 36.2% of packs (n=380) and the mean count (\log_{10} cfu/g) was 2.96 ± 1.47 . The distribution of counts is shown on Fig 5.

Figure 5. Distribution of *Enterobacteriaceae* counts (\log_{10} cfu/g) found in RTE meats (n=1049). Values above 4 are unsatisfactory, comprising 9.3% of packs.



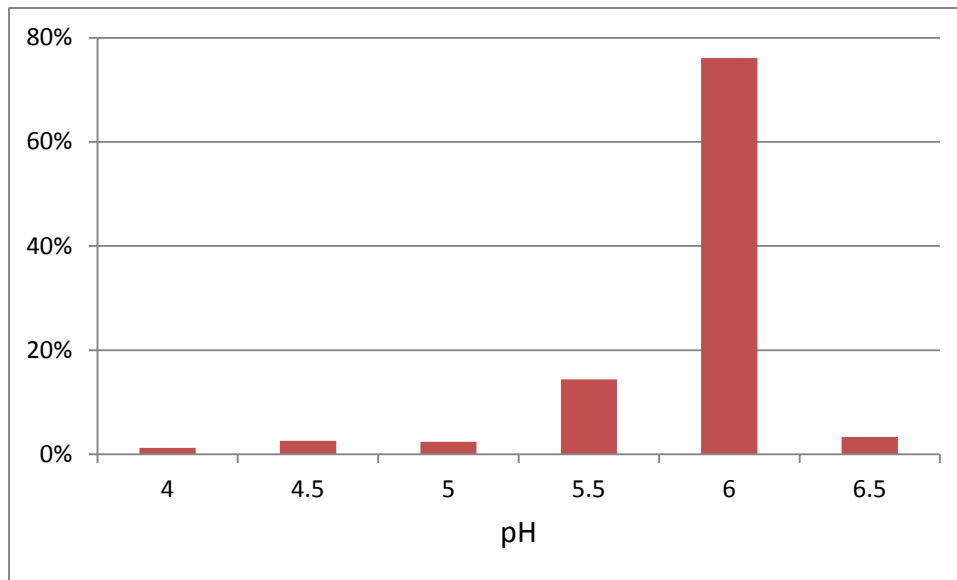
3.6 Presence of *Escherichia coli* in RTE sliced meats in SME

E. coli were enumerated from five samples (0.48%), two of which had levels of 100 cfu/g and were therefore unsatisfactory (HPA, 2009). The other samples yielded 5, 10, and 50 cfu/g.

3.7 pH

The mean pH of the samples was 6.09 ± 0.38 , and the distribution is shown on Fig. 6 below. Almost 80% of samples had a pH of 6.0 or greater and some strains of *L. monocytogenes* can grow at pH levels as low as 4.1 (Shabala et al. 2008).

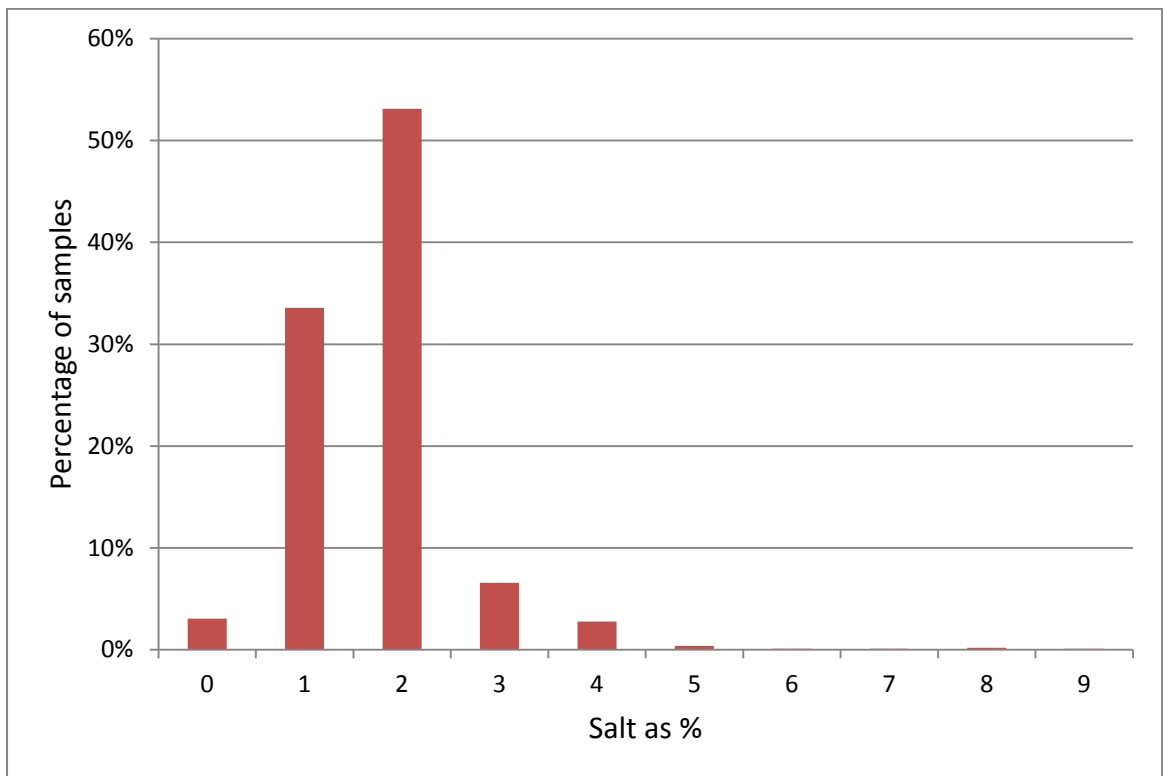
Figure 6. Distribution of the pH in RTE meat samples (n=1049). A value of 4 on the x-axis indicates the number of samples with $\text{pH} \geq 4 < 4.5$.



3.8 Salt content

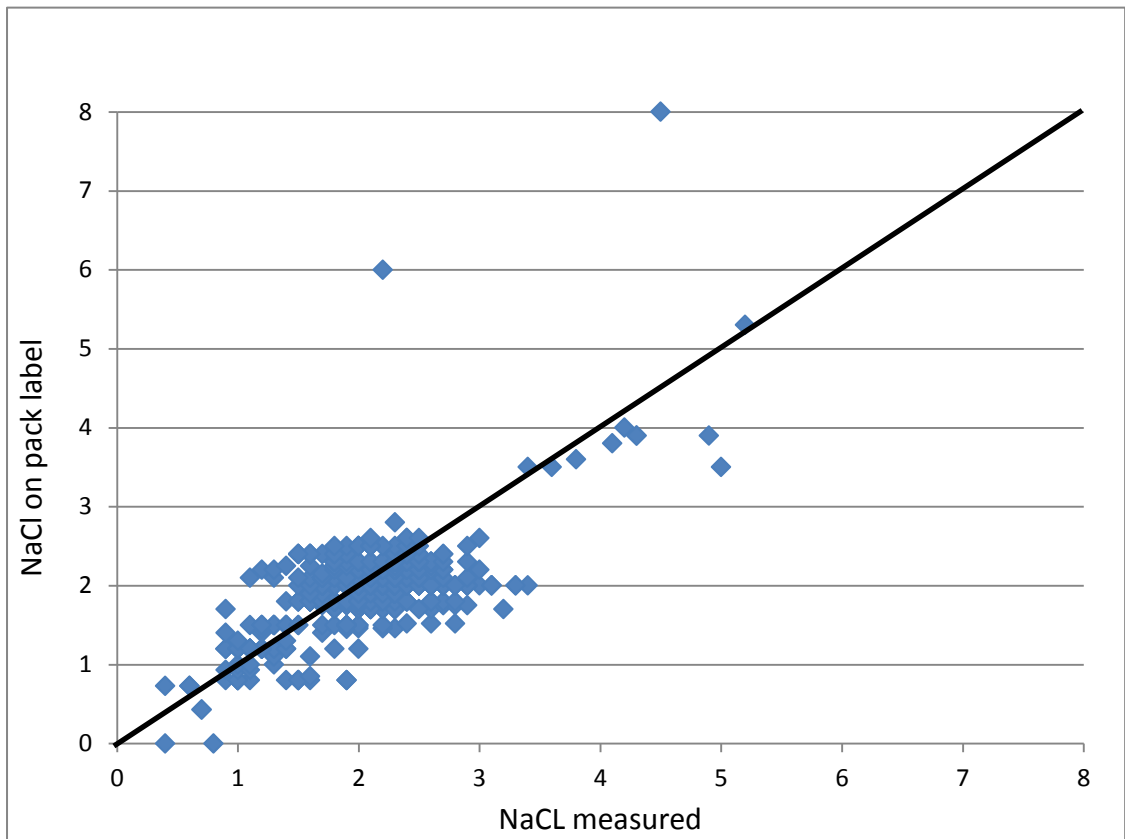
The mean salt (sodium chloride) content in the samples was (g/100g) 2.07 ± 0.82 , and the distribution is shown on Fig. 7, below. Almost 90% of samples had a salt content of less than 3%.

Figure 7. Distribution of salt content in samples as g/100g in 1048 samples. A value of 0 on the x-axis indicates the number of samples with salt content, $\geq 0 < 1$.



Retrospectively, the salt content displayed on the packaging was compared to that measured in the laboratory. Overall, salt levels could be obtained from 609 packs and for these packs the mean salt content, as determined in the lab, was 2.07% whilst that calculated from the level displayed on the pack was 1.96%. The trend for declared salt levels to be below that shown on the pack labels is illustrated in Fig. 8.

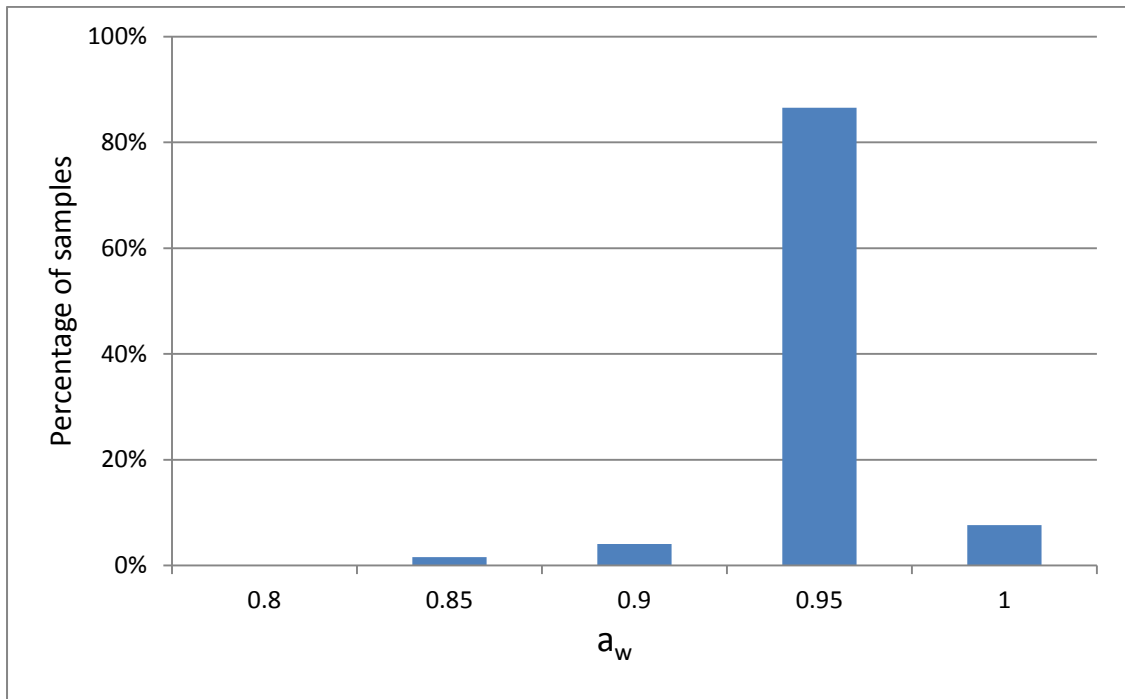
Figure 8. Comparison of salt levels found by laboratory analysis with those declared on the pack label. The line drawn shows equivalence, and the trend is for points to fall below the line, indicating that the measured salt level is greater than that declared.



3.9 Water activity

The mean a_w of the samples was 0.98 ± 0.02 and the distribution is shown on Fig. 9, below. The a_w of the samples was equal to, or greater than, 0.95 in 94.2% of samples ($n=988$).

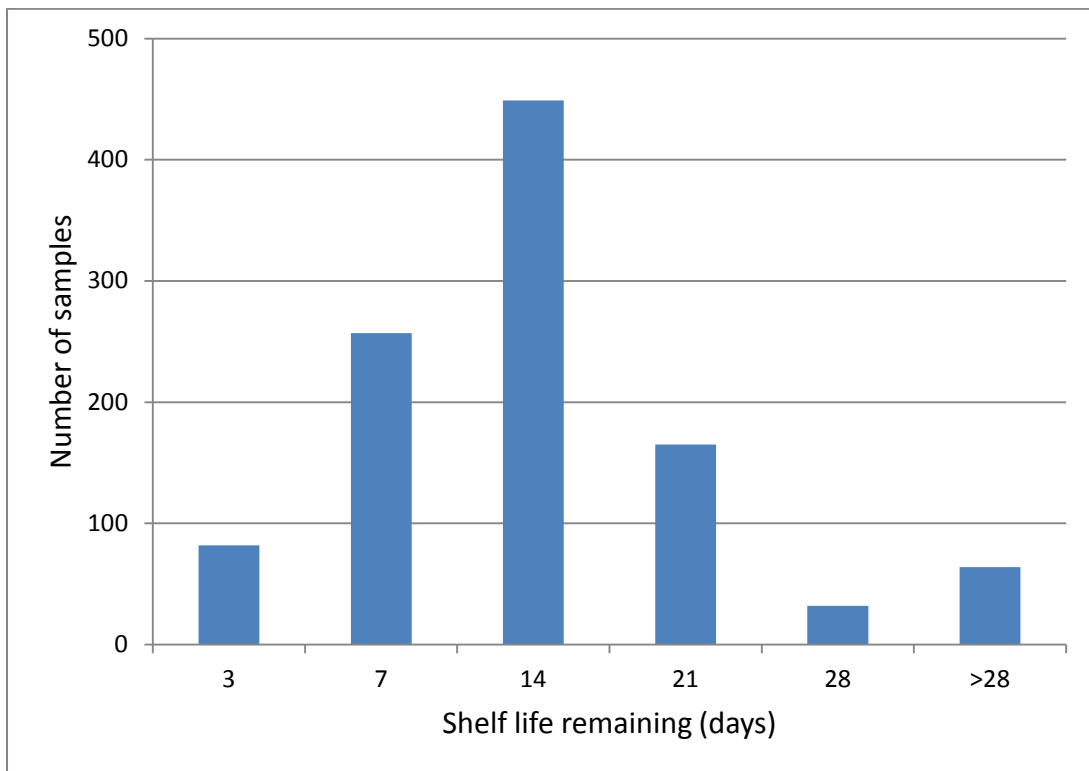
Figure 9. Distribution of water activity (a_w) in samples ($n= 1049$). A value of 0.9 on the x-axis indicates the number of samples with $a_w \geq 0.9 < 0.95$.



3.10 Shelf life

The remaining shelf life of samples was calculated as the time from the date of sampling until the 'Use by' date. The mean remaining shelf life was 13.8 days, and 75.1% of samples had 14 days or less of shelf life remaining. Fig. 10. Overall, 480 samples had a shelf life greater than 10 days, and 180 of these had water activities greater than 0.97. Only three of the latter had a pH of 5 or less and/or a minimum salt level of 3.5%.

Figure 10. Distribution of remaining shelf life of samples on day of purchase. A value of 7 d indicates the number of samples with remaining shelf life $> 3 \text{ d} \leq 7 \text{ d}$.



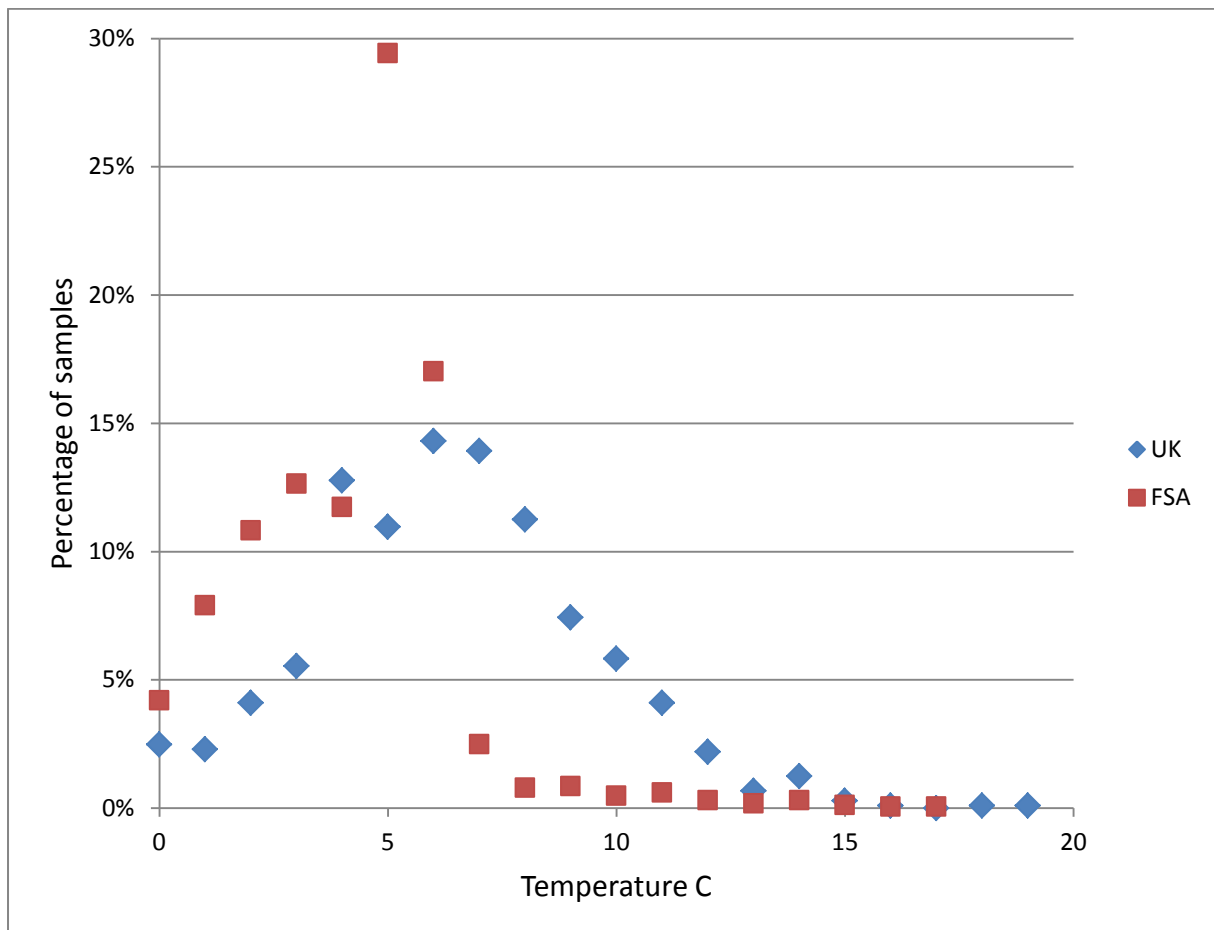
4 DISCUSSIONS

The project aimed to determine the prevalence of levels of microbiological contamination, especially *L. monocytogenes*, in RTE sliced meats available in SME retail outlets in the UK, and to provide an indication of potential underlying risk factors associated with these products. The growth of *L. monocytogenes* can be affected by temperature, pH, a_w , atmosphere and whether the substrate is solid or liquid (Koutsoumanis et al 2004; Miconnet et al. 2005). The interplay between these factors means that the rate of growth of *L. monocytogenes* observed, under similar storage conditions, on different products will differ due to the intrinsic properties of the foodstuffs (Beumer et al. 1996, Grau et al. 1992). The nature of the specific strain(s) of *L. monocytogenes* growing on a substrate will also affect the growth seen (Nufer et al , 2007; Shabala et al. 2008), as will the conditions they experienced prior to inoculation (Dykes, 2003).

In the majority of the samples examined in this study the pH, salt concentration and water activity of samples was in a range where growth of *L. monocytogenes* could take place, Figs 6, 7, and 9, based on calculations of the USDA growth model (<http://ars.usda.gov/Services/docs.htm?docid=11550>). However, due to the complexity of food matrices, combined with the wide range of contaminating strains of *L. monocytogenes*, such predictions must be interpreted with caution (Koutsoumanis and Angelidis 2007). One of the most significant factors affecting the growth of *L. monocytogenes* is product temperature (Buchanan et al. 1989; Buchanan and Phillips, 1990; Buchanan and Klawitter, 1991; Koutsoumanis and Angelidis, 2007), and growth has been reported as occurring at -0.4°C (Walker et al. 1990). Most samples were therefore held at temperatures (Fig. 4) where growth of *L. monocytogenes* can occur. Furthermore the growth rate of *L. monocytogenes* has been shown to increase significantly as the product temperature rises above 5°C (Tienungoon et al. 2000; Wijtzes et al. 1993).

Industry guidelines for chilled foods recommend that these products should always remain below 5°C (Goodburn, 2005), but 71.3% of samples collected in this study exceeded this guideline. Further, there is a legal requirement (EC Regulation 852/2004) to keep cooked meats below 8°C (Anon, 2007b) and almost a third of samples (32.7%) equalled or exceeded this temperature. Comparison with the previous UK-wide FSA study, B18024, (Anon. 2007a), which also sampled pre-packed cooked sliced meats, indicates the extent of the poor temperature control measured during this study, Fig 11. For the 2007 survey RTE pre-packed sliced meats ($n=1366$) were mainly purchased in supermarkets, and had a mean sample temperature of 4.4°C , with 50.4% of samples below 5°C , and only 2.4% exceeding 8°C .

Figure 11. Distribution of temperatures measured on RTE meats sampled in SME during this survey (UK) in comparison with those (FSA) found in 2007 in supermarkets in a previous survey (FSA B18024).



Further, a European baseline study on the prevalence of *L. monocytogenes* in RTE products (EFSA, 2013) sampled 3,530 packaged heat-treated RTE meat products in 27 countries, and the temperature of the products on display was measured. The mean temperature measured was 3.71°C (SD 1.78°C), and less than 15% of samples exceeded 5°C, indicating that refrigeration of RTE meats was apparently better than was seen in the current study, or the previous UK study.

Therefore, this study determined that there was widespread and frequent display of RTE meats above the guideline temperature of 5°C in SME retailers in the UK. Although not a formal part of this study, some thermographs were taken to verify the relatively high temperatures noted during sampling, and this work is illustrated in Appendix 2.

The products examined in this study, those reported from the previous FSA study (B18024) and the EFSA (2013) study, were all in sealed packs hence the microflora found in the products was derived from that present in the products at the time of packing. Since packaging integrity was a requirement for a sample to be acceptable for testing no subsequent contamination was possible, therefore the bacteria detected in these products were indicative of contamination in the processing plant. However, in

terms of microbial numbers reported, it should be noted that the EU survey results were measured at the end of the product's shelf life, following storage at a mean temperature of 4.51°C. This is a confounding factor in comparisons with previous surveys as Sagoo et al (2007), for example, studied packaged RTE meats in the UK and stored them until the end of shelf life, in their case at 6°C ± 2°C. In the current study the mean sample temperature was 6.80°C, and the mean remaining shelf life was approximately 14 d (Fig 10), indicating that there was significant potential for microbial growth before the end of the designated shelf life.

The presence of *Enterobacteriaceae* in cooked products implied either inadequate thermal processing, or contamination after the critical control point of thermal processing (Sagoo et al. 2007). Over a third of the samples analysed (36.2%) in the current survey contained *Enterobacteriaceae* (Fig. 5) and 9.3% of samples failed national guidelines i.e. *Enterobacteriaceae* counts exceeded 10⁴ cfu/g (HPA, 2009). Further, continued display could have led to microbial growth resulting in more unsatisfactory samples. These results contrast with the results of FSA study B18024, where only 8.2% of pre-packed cooked sliced meat samples yielded *Enterobacteriaceae* and 0.8% were unsatisfactory. In an earlier UK study of RTE meats, undertaken in 2003, Sagoo et al. (2007) reported similar results to those found in this study; 33% of RTE meat samples (n=2,981) yielded *Enterobacteriaceae*, and 7.6% of packs were unsatisfactory due to having >10⁴ cfu/g *Enterobacteriaceae*. However, once purchased these samples were stored (6°C ± 2°C) until the 'use-by' date, i.e. above the recommended limit of 5°C. In view of the different sample handling protocols direct comparisons with the results of Sagoo et al. (2007) are not possible, although wider consideration of the results of that study similarly imply poor processing plant hygiene.

The effects of storing RTE meat samples until the end of shelf life on the detection of *L. monocytogenes* was investigated by Little et al. (2009) who in 2006-2007 bought 684 duplicate packs of RTE meats and analysed one sample shortly after collection and stored the other at 6°C ± 2°C until the 'use-by' date. *L. monocytogenes* was detected in 3.7% of the former samples and 4.2% of the latter, showing that storage at temperatures above industry guidelines gave rise to an additional 0.5% in total detections of *L. monocytogenes*. Since the packs were sealed the increase in detections would have resulted from growth of *L. monocytogenes* from numbers below the limit of detection (theoretically 1 cell in 25g) to detectable numbers. Therefore storage, at a temperature which exceeded industry guidelines, caused a reduction in the microbial quality of products due to bacterial growth, as would be expected.

Comparisons of retail surveys, as discussed above, are complicated by several confounding factors. Some studies attempt to determine 'worst case' levels of *L. monocytogenes* and therefore samples are stored until the 'use-by' date. There is no consensus on the storage temperature used for such studies, or the precision with which the storage temperature should be maintained. The type of premises sampled will also affect the results obtained since large supermarkets have well maintained cold chains and will define product processing conditions to ensure the packaged product that they sell is unlikely to harbour human pathogenic micro-organisms. The low prevalence of *L. monocytogenes* in packaged RTE meat also means that large numbers of samples are required to be examined to precisely determine prevalence. Table 2 shows the results of a limited number of surveys of RTE meats.

Table 2. Reported prevalence of *Listeria monocytogenes* in pre-packed ready to eat meats. * indicates that samples were stored until the end of their shelf-life prior to analysis.

Year samples taken	Number of samples	Prevalence of <i>L. monocytogenes</i>	Reference
2003	2,981	5.5%*	Sagoo et al. (2007)
2006-2007	684	3.7%/4.2%*	Little et al. (2009)
2007	1,366	0.59%	FSA B18024
2010-2011	3,530	2.07**%	EFSA (2013)
2012	1,049	3.8%	This study.

In the survey of thermally processed, packaged RTE meats undertaken in 2010-2011 in 26 EU countries plus Norway (EFSA 2013), samples were stored at 4.51°C (SD 1.43 °C) for the duration of the shelf life, prior to analysis. As can be seen the prevalence determined was almost half of that found in this survey.

In the current study, the prevalence of *Listeria monocytogenes* was 3.8% (CI: 2.6% - 5.0%), whilst the previous study (B18024) found 0.59%. However, it should be noted there were detail differences in the analytical methods used in the respective surveys. However, one major difference between the two surveys was that the current study sampled exclusively SMEs whilst B18024 sampled mainly in large supermarkets. This survey found that sample temperatures were unacceptably high in most samples, and higher than those reported in both B18024 and by EFSA (2013). Such failure to meet industry temperature guidelines, and even legal requirements, would benefit the survival and growth of *Listeria monocytogenes*, if it were present already in the packaged meat.

Overall, it was noted that 60.3% of *Listeria* positive samples came from only six processors who had produced 15.8% of all samples tested. One of these processors produced 6.4% of all samples, but 17.8% of the total found to be *Listeria* positive. In addition, it was noted that 82% of the samples either had *Listeria* spp. or *Enterobacteriaceae* present. Thus it would appear that a marked reduction in the prevalence of *Listeria*, and *Enterobacteriaceae*, could be made by targeted interventions to improve production conditions in a small number of producers.

5 Conclusions

Overall, this study established that in the SME premises visited most pre-packaged RTE meats were being displayed at temperatures in excess of industry guidelines, and even legal requirements. Adequate refrigeration, i.e. below 5°C, is required to minimise microbial growth and failure to do so can permit the growth of bacteria, including the pathogen *L. monocytogenes*, during retail display. As RTE products are specifically intended to be consumed without any heat treatment, or other intervention likely to reduce the population of such organisms, the observed inappropriate refrigeration could increase the possibility of foodborne illness, especially among higher risk sections of the population.

Since only pre-packed RTE products, with intact packaging, were analysed the bacteria found in this study were incorporated in the processing plant, and are therefore indicative of hygiene problems in that environment. Samples from most (73%) of the 96 processors identified in this study did not contain any *Listeria*. However, on average, 25% of samples produced by the remaining 23 processors (representing 21% of the total sample population) were found to contain *Listeria* spp. Thus a minority of processors were responsible for the majority of products contaminated with *Listeria* spp. Where possible, processors identified as producing unsatisfactory products will have to be informed of the quality of their product, as determined in this study, and assistance provided by the relevant public bodies to ensure hygienic production conditions are instituted.

To reduce the prevalence of *Listeria* in RTE meat products on sale in SME retailers in the UK it will first be necessary to train retail staff in production premises to ensure a hygienic product is always produced. In the SME outlets staff should be trained in the appropriate control and maintenance of chilled display units. Monitoring to ensure compliance should be undertaken. Most manufacturers produced an acceptable product and maintaining the product temperature below the recommended 5°C until the point of sale will ensure risks to all consumers are minimised.

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7. Appendices

7.1 FSA requirement document

Requirement: Carry out a microbiological survey of pre-packed ready-to-eat sliced meats at retail in small to medium sized enterprises (SMEs)

Background

The FSA's Foodborne Disease Strategy (FDS) for 2010-2015 (see link below) proposes a pathogen-specific approach to reducing foodborne disease rates in the UK and *L. monocytogenes* has been identified as one of the priorities for action. This is because infections with this organism (listeriosis) are associated with a high rate of mortality. It is therefore important that sources of exposure to this organism are pinpointed and factors contributing to infections identified. The types of food in which listeria can be found are diverse and there are several key foods that have been implicated in the transmission of listeriosis, including cooked sliced meats.

Objectives

The primary objective of this survey is to determine the prevalence and levels of contamination in ready-to-eat cooked and cured sliced meats at retail sale in small to medium sized enterprises (SME's). The previous FSA survey of retail cold sliced meats with particular reference to *L. monocytogenes* was based on market share and therefore the majority of samples were collected from the large retailers. A recent HPA study of human listeriosis in England (2001-2007) found that the study cases (particularly the elderly who are a high risk group) were more likely to purchase foods from smaller convenience stores than the general population and so this survey will focus on SME retailers. This survey will also cover fermented and cured meat products that were excluded from the FSA survey.

Scope

The contractor will ensure the following are covered by this survey:

Samples will be tested for *L. monocytogenes* and other *Listeria* spp. (detection and enumeration). Samples will also be tested for the hygiene indicator organisms *Escherichia coli* and *Enterobacteriaceae* (detection and enumeration).

Samples should also be tested for physical characteristics, such as salt content, water activity and pH. The storage temperatures in the SMEs at the time of sampling should be recorded, as should a range of factors such as price, 'use-by' date and storage instructions.

It should be noted that this is intended to be a focused survey to determine prevalence of contamination in different RTE sliced meats to provide an indication of potential underlying risk factors associated with these products on sale in SMEs. It is not intended that this survey will investigate seasonality.

7.2 Thermal imaging of chillers.

A thermal imaging camera was used to obtain temperatures from a wide area within chill cabinets. Fig 11a and 11b show the elevated temperatures of the fluorescent tubes illuminating such cabinets, whilst Fig 12a and 12b show how many packs on the shelves are at similar temperatures



Fig 11 a



Fig.11b. Spot temperatures in typical chill cabinets showing the relatively high temperature of the illumination strip, over 30°C in both cases.



Fig 12a.



Fig 12b. Spot temperature distribution across several products showing how elevated temperatures are not restricted to just a few samples. The trend for recessed product to be cooler can also be seen.

7.3 Methodology of analyses.

Enumeration/ detection of *Enterobacteriaceae* in foods.

PROCEDURE

Method is based on based on BS ISO 21528-2:2004.

1. PREPARATION OF THE SAMPLE:

Correct preparation of the sample prior to testing is essential to ensure sample is representative of the original batch or load tested.

2. TEST PORTION AND PREPARATION OF PRIMARY DILUTION:

Primary and successive dilutions shall be prepared if required using buffered peptone diluent. A number of dilutions shall be prepared with the aim that the final dilution on testing shall give countable results.

The diluted sample thus prepared shall be either mixed thoroughly by shaking 25 times in about 10 seconds through an arc of around 300 mm, or using a vortex mixer, or for solid/semi-solid/powder samples stomached for 1 minute, to give the primary (10^{-1}) dilution. Using a 'fresh' pipette/pipettor tip 1 ml \pm 0.1 ml aliquot of the primary dilution shall be transferred into another tube containing 9 ml \pm 1 ml diluent, avoiding contact between the pipette/pipettor tip and the diluent. Further dilutions shall be similarly prepared as required.

3. INOCULATION

For each portion of the sample 1 ml of solution shall be transferred to a sterile 90 mm petri dish (in duplicate). The plates shall be labelled to identify the portion of sample they were taken from. 15 ml of violet red bile glucose agar (VRBGA) at a temperature of 49°C \pm 1°C shall be added to each petri dish and immediately gently mixed by swirling the dish with five clockwise and five anticlockwise circular movements.

Once the agar has set, each agar plate shall be overlaid with a further 10 ml VRBGA at a temperature of 49°C \pm 1°C. Once the overlay has set, the plates shall be inverted and incubated aerobically at 37°C \pm 1°C for 20 hours \pm 2 hours.

After incubation each set of duplicate plates shall be examined for colonies characteristic of *Enterobacteriaceae* (purple colonies 1 - 2 mm in diameter). All characteristic colonies on each plate shall be counted and the arithmetic mean of the duplicate plates taken. After counting the colonies, characteristic colonies shall be taken at random from the agar plates, the number being at least the square root of the colonies counted. The colonies shall be subcultured onto a nutrient agar plate and incubated aerobically at 37°C \pm 1°C for 20 hours \pm 2 hours.

4. CONFIRMATION OF SUSPECT ENTEROBACTERIACEAE

The oxidase test and a glucose fermentation test shall be performed on each of the subcultured colonies. Colonies which are oxidase-negative and glucose fermentation-positive shall be considered to be *Enterobacteriaceae*. If not all of the colonies prove to be *Enterobacteriaceae*, the total count shall be reduced in proportion.

Enumeration of *Escherichia coli* in foods.

PROCEDURE

Method is based on based on BS EN ISO 16649-2:2001.

SAMPLE PROCESSING

Sample preparation and dilution

Following the procedure described in ISO 7218, prepare a 10⁻¹ homogenate in either peptone saline diluent (PSD) or buffered peptone water and further decimal dilutions as required in PSD.

Inoculation and incubation

Inoculate 0.5 mL of 10⁻¹ homogenate onto the centre of a pre-dried BCIG agar plate. Using a sterile spreader carefully spread the inoculum to cover the surface of the medium trying not to touch the sides of the petri dish. Allow the inoculum to be absorbed into the plate and place in an automatic cycling incubator at 37°C for 4 ± 1 hours and then at 44°C for 18 ± 2 hours. The incubation temperature shall not exceed 45°C. Where it is necessary to detect 10cfu/g use two plates, each inoculated with 0.5ml of 10⁻¹ homogenate.

Counting of colonies

Examine the BCIG agar plates for blue colonies (β-glucuronidase positive). Using plates containing up to 150 colonies count and record the number of typical colonies.

Confirmatory tests

No confirmatory tests are needed. Identification is based on the growth on tryptone bile agar at 44°C and a positive β-glucuronidase reaction (blue colonies).

Control cultures

Positive control: - *E. coli* NCTC 9001; Negative control: - *K. aerogenes* NCTC 9528

Detection and enumeration of *Listeria spp.*, including *Listeria monocytogenes*.

Detection method is based on BS EN ISO 11290-1:1997. Enumeration method is based on based on BS EN ISO 11290-2:1998.

PROCEDURE

1. TESTING. Samples submitted for testing shall be examined on the day of receipt, if possible. Those not examined on the day of receipt shall be stored in a 2° to 5°C refrigerator/chill store for not more than 1 day.

Positive and negative controls, shall, where appropriate, be incorporated with each run of test samples analysed. The positive and negative controls used in this procedure shall be as follows: -

Analysis: -	Positive Control: -	Negative Control: -
Detection of <i>Listeria</i> spp.	<i>L. monocytogenes</i> (NCTC 11994)	<i>Staphylococcus aureus</i> (NCTC 6571) Neg. control for Gram reaction: <i>Salmonella</i> Lindi (NCTC 08494)

The lower limit for detection for *Listeria* using the following method has been determined as ≥ 5 cells.

2. ENUMERATION METHOD

On day 1, examination of samples shall be carried out by weighing $25 \text{ g} \pm 1 \text{ g}$ of representative test portion using a calibrated balance, into a stomacher bag. Add $225 \text{ ml} \pm 5 \text{ ml}$ of Fraser Broth without selective supplements to the test portion. Blend for a 'minute' to ensure an even distribution of contained microorganisms. Let this initial suspension stand for $1 \text{ hour} \pm 5 \text{ minutes}$ at $20^\circ\text{C} \pm 2^\circ\text{C}$. Additional dilutions (if required) of the initial suspension shall be performed after this time. $100 \mu\text{l}$ of well-mixed initial suspension shall be spread, in duplicate, onto ALOA agar and Palcam agar plate(s) using a sterile spreader. ALOA agar plates shall be incubated aerobically at $37^\circ \pm 1^\circ\text{C}$ for 24 to 48 hours. Palcam agar plates shall be incubated aerobically, or microaerophilically at $37^\circ \pm 1^\circ\text{C}$ for 24 to 48 hours.

On completion of the period of incubation the ALOA agar and Palcam agar plates shall be examined for the presence of colonies that, from their appearance, are considered to be presumptive *Listeria* species. Count selective plates with less than 150 typical colonies.

3. DETECTION METHOD

Selective Repair/Recovery (Primary) Enrichment

On day 1, liquid test samples shall be thoroughly mixed by shaking 25 times within about 10 seconds through an arc of around 300 mm. Examination of liquid samples shall be carried out by pipetting 25 ml \pm 1 ml of well mixed test portion of each sample into 225 ml \pm 5 ml of half Fraser broth complete with supplements. The enrichment medium/test sample mix shall be thoroughly mixed and shall be incubated at 30° \pm 1°C for 22 to 26 hours.

On day 1, examination of powdered/dried; viscous; semi-solid; or solid samples shall be carried out by weighing 25 g \pm 1 g of representative test portion using a calibrated balance, into a stomacher bag. Add 225 ml \pm 5 ml of half Fraser broth complete with supplements to the test portion. Blend for a 'minute' to ensure an even distribution of contained microorganisms. Incubate the enrichment medium/test sample mix at 30° \pm 1°C for 22 to 26 hours.

Should there be a requirement to analyse frozen foods such product(s) shall be allowed to thaw at 2° to 5°C overnight before analysing according to the protocol described above.

Except in the case of powdered/dried food product samples the interval between mixing and sub-sample removal shall not exceed 3 minutes.

4. SECONDARY ENRICHMENT:

On day 2, after incubation of the initial repair/recovery enrichment (primary enrichment), 0.1 ml of the primary enrichment shall be pipetted into 10 ml \pm 0.5 ml Full Fraser Broth, contained in test tubes, complete with supplements. The contents shall be well mixed. This secondary enrichment shall be incubated at 37° \pm 1°C for 46 to 50 hours.

5. Isolation and presumptive identification

On day 2, 10 μ l of well-mixed 'overnight' selective repair/recovery enrichment broth (Section 3.2) shall be streaked onto ALOA Agar and Palcam Agar plate(s) using a sterile disposable loop. ALOA Agar plates shall be incubated aerobically at 37° \pm 1°C for 24 to 48 hours. Palcam Agar plates shall be incubated aerobically, or microaerophilically at 37° \pm 1°C for 24 to 48 hours. On completion of the period of incubation the ALOA Agar and Palcam Agar plates shall be examined for the presence of colonies that, from their appearance, are considered to be presumptive *Listeria* species.

6. On day 4, following completion of incubation of the secondary enrichment phase (Section 3.2.1), 10 μ l of well mixed enrichment shall be streaked onto ALOA Agar and Palcam Agar plates using a sterile disposable loop. ALOA Agar plates shall be incubated aerobically at 37° \pm 1°C for 24 to 48 hours.

Palcam Agar plates shall be incubated aerobically or, microaerophilically at $37^{\circ} \pm 1^{\circ}\text{C}$ for 24 to 48 hours.

7. On day 5 examine the ALOA Agar and Palcam Agar plates after approximately 24 hours incubation for typical *Listeria* colonies. If suspect colonies present proceed to Section 3.4. If no suspects present continue incubation for a further 18 to 24 hours.
8. On day 6 re-examine the ALOA Agar and Palcam Agar plates for typical *Listeria* colonies. If suspect colonies present proceed to Section 3.4. If no colonies typical of *Listeria* spp have been detected on either ALOA Agar or Palcam Agar plates at this stage of the procedure, no *Listeria* spp. have been detected in the sample.
9. CONFIRMATION
Three colonies of presumptive/suspect *Listeria* species (Section 3.3) from each presumptive 'positive' selective agar plate shall be sub-cultured onto 2 separate tryptone soya yeast extract agar (TSYEA) plates, 1 of which shall be a thinly poured (12ml) TSYEA plate. The plates shall be incubated at $37^{\circ} \pm 1^{\circ}\text{C}$ for 18 to 24 hours. If there are fewer than three presumed *Listeria* colonies on a plate then all colonies shall be selected for confirmation. The identity of these isolates shall be confirmed by means of appropriate morphological, physiological and biochemical tests.
10. CARBOHYDRATE/BIOCHEMICAL REACTION:
Identification kits acknowledged as being of acceptable standard e.g., API-LIST Biomerieux, shall be used to assist in the confirmation, using carbohydrate/biochemical reactions, of suspect *Listeria* species isolated, and to speciate the *Listeria* strain(s) isolated, including *Listeria monocytogenes*.

Biochemical and other reactions of *Listeria* species

	Tumbling motility	Gram stain	β haemolysis	Mannitol*	Rhamnose	Xylose	N-acetyl- β -D mannose	Ribose*
<i>L. monocytogenes</i>	+	+	+	-	+	-		
<i>L. innocua</i>	+	+	-	-	V	-		
<i>L. ivanovii</i> <i>var ivanovii</i>	+	+	+	-	-	+	-	+
<i>L. ivanovii</i> <i>var Londoniensis</i>	+	+	+	-	-	+	+	-
<i>L. seeligeri</i>	+	+	+	-	-	+		
<i>L. welshimeri</i>	+	+	-	-	V	+		
<i>L. grayi</i>	+	+	-	+	-	-		

+ = positive reaction
 - = negative reaction
 V = variable reaction
 (*) = required occasionally

Determination of the water content of RTE meat.

Method conforms to the British Standard method BS ISI 21807:2004.
Measurement of water activity is achieved by the direct determination of the equilibrium water-vapour pressure in a closed system.

PROCEDURE

1. Ensure that the external 9V adapter is plugged into the back of the meter.
2. Insert the plug of the adapter into the mains socket and switch the socket on.
3. Switch on the meter by pressing the red ON/OFF button on the right of the control panel.
4. If necessary, plug the probes into the pack panel and secure with the locking ring.
5. The water activity meter is kept in an incubator cabinet set at a temperature in the range 18 - 25°C to maintain a constant temperature during measurement.
6. Ensure that the test material is at the same temperature as the probe (preferably allow to equilibrate for 1 hour in the incubator) before starting measurements.

Full Calibration:

1. Use the four standards detailed in 3.1.
2. Place an aliquot of the first standard solution in four disposable plastic containers. The container should be about 1/3 full. DO NOT FILL ABOVE THE FILL LINE. N.B. Standard solution or sample material should not be allowed to come into contact with the probe.
3. Place a sample container into each of the four sample holders. Record which standard is in each sample holder 1 to 4.
4. Place the probe on top of the sample holder and press down the arm until it clicks firmly into place thus forming a tight seal.
5. Check that the probe is switched on – a red light will flash. If the light is not lit, press the red button on top of the probe to switch it on.
6. Choose the quick mode (Q). To do this press the MENU button on the front panel. Scroll through the menus using the up and down arrows to find MODE. Press the ENTER button. Scroll through the modes using the up and down arrows to find the desired mode. Press the ENTER button to exit the menu. The mode chosen will be memorised and will be used automatically on the next occasion.
7. Press the Enter button to start measuring
8. Leave until the reading has stabilised reading.
9. Use the first reading for each probe as a “warm up” measurement. Do not record.
10. Take a second reading for each probe using the same standard solution. To do this release the lever and lift the probe off the sample holder for about five seconds. Replace the probe and reseal using the lever. Allow to equilibrate. Record the result
11. Repeat 10 twice more thus giving three standard readings for each probe.
12. The three results should not vary by more than 0.005 units from the expected result for the solution. If the readings are acceptable return the aliquot of standard solution to its flask.
13. If the repeatability of the readings is not acceptable then repeat 10.

14. Repeat the procedure for each of the standards until three readings are obtained for each standard for each probe.
15. Prepare a graph of concentration of NaCl (x) against reading (y) and show the $mx + c$ equation on the graph.

Sample treatment

1. Sample preparation: - do not homogenise. Tear some pieces off the original sample to fill the sample container about 1/3 full. Do not allow the sample to come into contact with the probe.
2. Once the daily calibration has been completed carry out the same procedure as detailed above for the samples.
3. Three readings should be recorded for each sample. The three results should not vary by more than 0.005 units.

Results

Record a mean of the three readings for standards and samples.

Determination of the pH of cooked meat.

Method conforms to the British Standard method BS 4401-9-1975. ISO 2917-1974.

PROCEDURE

Take three measurements on the same sample.

Calibrate the pH meter using buffers pH 4 and 7.

1. Proceed from a representative sample of at least 200g.
2. Determine the pH immediately or store the sample in such a way that pH changes will be restricted to a minimum.
3. Pass the sample twice through the meat mincer (pore size <4mm) and mix thoroughly.
4. Use enough of the test sample to immerse the pH probe sensor.
5. Homogenise the sample with an equal mass of water for at least one minute – use a timer.
6. Place the pH probe in the sample.
7. When a constant value has been reached record the pH to the nearest 0.05pH unit.
8. Carry out three measurements on the same test sample.
9. Wipe the probe with tissue and wash with water.
10. Store the probe in 3M KCl.

Result

Take as the result the mean of the three values, provided that the difference between the extreme values obtained does not exceed 0.15 pH unit. If the repeatability requirement is satisfied take the mean of the three values as the result.

Determination of salt by chloride content (potentiometric method)

Method conforms to the British Standard method BS 4401-16: 1996 for the determination of the chloride content of cooked meat.

1 PROCEDURE (carried out in duplicate)

Titration Curve

1. Pipette 25mls of sodium chloride solution into a 250ml beaker and 25mls distilled H₂O. Using a 50ml measuring cylinder add 50ml of the dilute nitric acid solution.
2. Fill a 50ml burette with the silver nitrate solution, add a magnetic flea to the beaker and insert the silver nitrate electrode. Titrate with silver nitrate solution adjusting increments with the rate of change in potential to obtain an accurate plot of change in potential in millivolts (Y axis) against volume (ml) of silver nitrate solution (X axis).
3. Add a total of 50ml silver nitrate solution to obtain a complete titration curve.
4. Determine the inflection point by drawing 2 straight lines with a 45° slope with respect to the 2 axes and tangent to the titration curve at 2 points of greatest curvature.
5. The inflection point is at the intersection of the titration curve with the line drawn parallel to, and midway between, the other 2 lines.
6. Use the inflection point as the endpoint in titrating the sample solution.
7. Recheck the endpoint potential occasionally with respect to the junction potential developing at the reference electrode.
8. Redetermine the endpoint if electrode or pH meter is replaced.
9. From the volume of silver nitrate solution used, calculate the concentration and adjust to 0.0856 mol/l.

Sample determination

1. Homogenise sample and store in airtight container in fridge/freezer (ensure sample will not deteriorate prior to analysis).
2. Weigh to the nearest 0.1g, 50g of sample and transfer to a 1000ml blender jar, add 450ml distilled H₂O.
3. Blend for 1min at a low speed and then for 1-2min at higher speed to ensure the formation of a uniform suspension.
4. Weigh a series of numbered 250ml beakers.
5. Pipette immediately after blending using 50ml bulb pipette, 50ml of suspended solution into one of the weighed 250ml beakers noting the beaker number.
6. Determine the mass of the test solution.
7. Using a 50ml measuring cylinder add 50 ml nitric acid solution.
8. Titrate with silver nitrate as described above.
9. Use a 10ml burette if chloride concentration is ≤ 1%.

Blank test

Carry out a blank determination, following instructions for sample determination above, substituting the distilled H₂O for the sample suspension.

1 Calculation Of Results

Chloride content

$$W_{\text{Cl}} = \frac{(V_1 - V_2) \times c \times 50 \times 58.44}{m_1 \times m}$$

W_{Cl} = chloride content of the sample, expressed as NaCl as a percentage by mass

V_1 = volume (mls) of silver nitrate solution used in the determination

V_2 = volume (mls) of the silver nitrate solution used in blank test

c = concentration (mols/l) of the silver nitrate solution

m_1 = mass (g) of the test solution

m = mass (g) of the test portion

Report result to the nearest 0.1% (m/m)

8 Appendix
FSA Draft protocol.